JOURNAL OF AGRICULTURAL RESEARCH

VOL. XIX WASHINGTON, D. C., MAY 15, 1920 NO. 4

HALO-BLIGHT OF OATS[1]

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INTRODUCTION

The present paper is a description and discussion of a bacterial disease of oats which has been the subject of investigation by the writer for the past three years. This "halo-blight" is a disease which occurs to at least some extent each year throughout the oat-growing sections of the central and eastern States and becomes of economic importance during certain seasons when weather conditions are particularly favorable to its development.

During the season of 1918 field observations and specimens of diseased plants from widely separated sections of Wisconsin showed that this disease occurred in practically all the oat fields of the State and was responsible for the abnormal condition prevalent in the early part of that season.

DESCRIPTION OF HALO-BLIGHT LESIONS

The halo-blight is most conspicuous on the leaves (Pl. C; 26), although it may occur on leaf sheaths and glumes (Pl. 29). Typical well-developed lesions of the disease are oval chlorotic spots ½ to 2 cm. or more in diameter about points of infection which consist of gray-brown collapsed tissue measuring from 1 to several millimeters in length. The halolike border is at first only slightly lighter green than the surrounding tissue, but as it becomes older it loses more of its green color and forms oval yellow halos about the central infection areas.

Lesions are first visible as light green oval spots 4 to 5 mm. in diameter with central sunken points of infection at first evident only on one side of the leaf. This center of infection increases slowly in size, penetrates

[1] The greater part of this work was carried on at the University of Wisconsin during 1917 and 1918 under the direction of Prof. L. R. Jones and was continued in the Pathological Laboratory of the United States Department of Agriculture during 1918 and 1819 under the direction of Dr. Erwin F. Smith. The writer also wishes to acknowledge the courtesy of the Boston Branch of the Association of Collegiate Alumnae whose research fellowship she held during the college year of 1917–18.

the leaf tissue, and in a day or two forms a gray or brown dry tissue from 1 to several millimeters in diameter, evident on both sides of the leaf blade. The halolike margin spreads rapidly, becoming uniformly lighter green to yellow or showing concentric markings (Pl. 26) of different shades of green and yellow. Occasionally these halolike margins are prolonged at one end into points (Pl. C) from 1 to several centimeters long. They may extend as yellow streaks through the center or along the margin to the tip of the leaf, but ordinarily they appear as oval spots, measuring 1 cm. or more in diameter. Marginal infections are common, forming crescent-shaped lesions. These halolike lesions are conspicuous and characteristic. Except in the central infection area the tissues remain turgid and have a normal appearance except for the paler yellowish color. There is no water-soaked margin about the halo as described by Wolf and Foster (*10*)[1] for similar lesions of the wildfire disease of tobacco, and the spots do not fall out of the leaves. Exudate does not occur in connection with the lesions. When several lesions occur on the same leaf they often coalesce and produce a general yellowing followed by a breaking across of leaf blades (Pl. C) or a shriveling and drying of tips and margins. During periods of warm, dry weather yellow haloed leaf tissue loses its turgidity and color and forms oval, gray-brown dead spots which on some leaves have narrow, brown margins and on others narrow, yellow halolike margins. Very rarely the dead tissue may assume a pinkish or reddish brown color. In separate lesions the oval outline of the dead halo persists, and even when the whole leaf becomes dry and brown, the original halo outlines may still be distinguished.

PREVALENCE AND GEOGRAPHICAL DISTRIBUTION

Personal observations in Wisconsin and specimens of diseased plants from Ohio, Illinois, Indiana, Minnesota, Tennessee, California, and Virginia have led to the conclusion that halo-blight is present in oat fields every season, scattered lesions occurring on the lower leaves more or less throughout the season and occasionally attacking the panicles. These lesions on the lower leaves are more or less hidden by the fresher upper leaves and so escape observation. Only under particularly favorable weather conditions does the blight develop sufficiently to attract attention or to do serious damage.

FIELD WORK IN 1918

During the season of 1918 weather conditions favorable to halo-blight prevailed in Wisconsin and parts of adjoining States, causing an unusually severe bacterial blighting. In the experimental plots, halo lesions began to appear on from 1 to 25 per cent of the young plants about the middle of May. By May 25 practically every plant showed

[1] Reference is made by number (italic) to "Literature cited," p. 172.

some spotting. During the last week in May and the first week in June all untreated plots looked yellowed or slightly browned when viewed from a distance. Practically every first leaf and half of the second leaves were yellowed and dead. Many leaves had yellowed, shriveled tips and margins, and single lesions were abundant on the upper leaves of many varieties.

Every field about Madison showed some blighting. Usually the brown, dead leaves were easily seen from the road, and 100 per cent of infection was not at all uncommon. Some fields south of Madison showed distinct yellow spots from a yard to a rod or more in diameter.

One field of oats near Monroe, Wis., visited May 29, was so badly blighted as to show from a distance a general yellowing with scattered patches of more marked yellow. Closer examination showed abundant halo lesions, every plant being infected. About 3 per cent of the plants were yellowed throughout, the outer leaves were water-soaked and dead, and some whole plants were stunted to such an extent that their recovery seemed doubtful. On the remaining 97 per cent of the plants the outer two to three leaves were collapsed and dead, and the others showed scattered halo lesions in varying stages of development. Where the blight was farther advanced the leaves were broken over and the tips shriveled and brown. Other leaves showed typical, conspicuous, isolated halo lesions which were central or marginal, covering one-half to the entire width of the leaf blade. The plants in this field showed no marked reddening. They had been badly beaten by recent driving storms. Two other oat fields in the vicinity showed a normal stand, but the halo-blight was abundant. No plants remained uninfected, but nevertheless none were stunted or entirely yellowed, and chances for recovery were much better than for the field described above. The blight was general throughout the section about Monroe, and the two fields last mentioned probably represented the average. This yellowed condition of oat fields in this section was first evident May 26 and was reported by a number of farmers.

From May 31 to June 2 oat fields were visited by the writer in five counties of southern Wisconsin. More than 130 fields were inspected, and every one showed halo-blight varying in amount from a fraction of 1 per cent to 100 per cent, the latter being much the more common. The amount varied not only in individual fields but also conspicuously in different counties.

In Jefferson County 26 fields were visited. The oats were about half grown. One-fourth of the fields showed only scattered lesions on the lower leaves—an infection of 1 per cent or less. About one-half of the fields showed a general spotting of the lower leaves on from 60 to 100 per cent of the plants. In some cases the infection was in patches from 2 to 6 feet in diameter, where every plant had all but the last one or two leaves badly spotted. A few fields showed general and heavy infection of 100 per cent of the plants. Even the upper leaves were spotted.

The lower leaves were mostly gone, but a general yellowing of the fields was not marked. Only one field was so seriously affected as to show heavy general blighting and large yellow spots 1 to 3 rods across. About 60 per cent infection of the lower leaves was typical for the fields throughout this section.

In Dodge County the halo-blight was much more abundant. Of the 37 fields visited all showed at least 20 per cent infection; 5 showed light infection—spotting of the lower leaves of 20 to 50 per cent of the plants. This infection, however, was evident from the road. Over half of these fields showed heavy infection—60 to 100 per cent—on at least the lower leaves, and yellowed spots in the fields. The plants in these yellowed spots had little normal green leaf area, and as many as 10 per cent of the plants were entirely yellow and stunted. About one-third of the fields showed 100 per cent infection of the lower leaves, the browned tips and margins showing plainly and often giving a brownish tinge to the fields. In two fields the lower two to three leaves were practically dead and the upper leaves so badly spotted as to give a general yellow color to the fields. In all fields visited in Dodge County blight was evident without a close examination and was sufficiently severe to threaten the crop if unfavorable weather conditions continued. New green leaves were just beginning to appear.

In Fond du Lac County, farther north, the plants were smaller—6 to 8 inches high—and the blight was not heavy in most fields. Seven fields showed only traces of blight on lower leaves—1 to 30 per cent. One showed 100 per cent infection on the lower leaves and another heavy infection—100 per cent—and a general yellowing of the field.

In Columbia and Sauk Counties 10 fields showed a normal blue-green color but had 20 to 100 per cent infection on the lower leaves. Ten other fields showed yellow spots or a general yellowing of the fields. This section was second to Dodge County in the amount of bacterial blight.

Reports and specimens of plants from 35 counties in Wisconsin showed that leaf lesions were general throughout the oat-growing sections of the State and that a single disease, the halo-blight, was responsible for the trouble. A similar condition was reported for the oat fields of southern Minnesota, Iowa, northern Illinois, and Indiana.

For several years previous to 1918 this bacterial blight was observed in Wisconsin oat fields, but there was never enough of it to attract particular attention. The cool, cloudy days and frequent rains of the 1918 oat season proved to be just the conditions necessary to favor the development and spread of the disease. The average rainfall for May, 1918, was 6.66 inches, or considerably more than the normal for that month and greater than for any year since 1892. At Madison there were only four clear days during the month, and at least four heavy rainstorms were accompanied by strong, driving winds especially favorable to the spread of the disease. During June the weather conditions were much

less favorable for the spread of the bacterial blight. The total precipitation in Wisconsin for June was 2.31 inches, or below normal, while the average temperature increased from 58.1° F. in May to 63.9° in June.

With this rise in temperature and decrease in rainfall reports came in of improved conditions in the oat fields. The new leaves which came out were unspotted, and by the last of the month all the fields had resumed a normal color and appeared to have almost completely recovered. The badly yellowed field near Monroe was visited again July 2. It had resumed a normal green color throughout with no halo lesions on the upper leaves and only scattered old lesions lower down. The stand was thin and the plants smaller than in adjoining fields. Neighboring fields were just heading out, but this field would be 10 days to 2 weeks late. Other fields showing yellow spots were reported to have resumed a normal color, but plants in spots previously yellowed were at least a week behind the others in development. This change of weather conditions in June came at an opportune time. Continued cloudy, rainy weather would undoubtedly have destroyed many plants and reduced the yield. As it was, reports for the two seasons of 1917 and 1918 show an increase per acre for the whole State of 2.2 bushels in 1918, but this increase would undoubtedly have been more than doubled but for the presence of halo-blight. Following the unusually severe bacterial blight of the early part of the season, blasting of panicles was also unusually abundant and general throughout Wisconsin oat fields during 1918. In extreme cases as many as 25 to 50 per cent of the spikelets in a head were undeveloped. Counts of 30 panicles in a severely blighted spot gave an average of 29 spikelets per panicle and 31 per cent blasting. Counts of 30 panicles from a part of this same plot not severely halo-blighted gave an average of 34 spikelets per panicle and 20 per cent blasting.

On six panicles sent in from Lincoln County the numbers of normal and blasted spikelets were as follows:

Panicle No.	Number of normal spikelets.	Number of blasted spikelets.
1	36	28
2	24	[a] 34
3	38	28
4	10	8
5	34	20
6	16	19

[a] Top blasted.

The blasted spikelets are mostly in the lower half of the panicle, but occasionally the upper half is blasted as in No. 2.

All the experimental plots showed considerable blasting and numerous empty spikelets. Counts of 36 panicles of Wisconsin No. 14 oats from treated seed showed an average of 11 per cent of the spikelets blasted,

varying from 0 to 30 per cent. Counts of 40 similar panicles showed 21 per cent of the spikelets blasted. Experiments carried on during the summer of 1918 indicate that this blasting is probably not due to the bacterial disease but to the unusual meteorological conditions which favored the development and spread of the bacterial blight.

BACTERIAL ISOLATION EXPERIMENTS

Oat plants showing typical lesions of halo-blight were collected from fields around Madison, Wis., and from other points in the State, from Tennessee, Urbana, Ill., Lafayette, Ind., Wooster, Ohio, Davis, Calif., and Arlington Farm, Va. Twenty-eight isolations were made from these lesions, and 36 isolations from halo lesions produced by inoculations in the field and greenhouse. Most of these isolations were from leaf lesions, but a few were made from lesions on glumes (Pl. 29).

The first isolations were made by washing the leaf tissue through 10 sterile water blanks, crushing on a sterile slide, transferring to broth, and plating from this broth suspension. Later isolations were made by dipping the tissue for a second in 95 per cent alcohol, then into 1 to 1,000 mercuric chlorid ($HgCl_2$) for one minute, washing through three sterile water blanks, and proceeding as in the earlier method. This later method proved to be more satisfactory, but a comparison of the results from both methods proved interesting.

From all these isolations, with the exception of two from glumes, typical white colonies of the halo organism were obtained. These appeared on potato agar in from 1 to 3 days. When the first method of isolation was used, without sterilizing the surfaces of the tissues, yellow colonies appeared on the plates with the white colonies in 25 per cent of the isolations from natural infections and in 22 per cent of the isolations from inoculation experiments. When the surfaces of the lesions were sterilized in mercuric chlorid for one minute no yellow colonies were obtained. Twelve isolations were made from natural infections, using mercuric chlorid; and a still larger number were made from lesions due to inoculation experiments. One set of isolations was made by placing the leaf tissue in the mercuric chlorid for only 30 seconds. This leaf tissue had been sprayed with a mixed culture of yellow and white organisms. Yellow colonies appeared on the plates with the white colonies, but the yellow colonies were not nearly so numerous as on plates poured from tissue which had not been sterilized If the tissue had remained in the mercuric chlorid for 60 seconds instead of 30 seconds no yellow colonies would have appeared. These yellow organisms appear to be surface saprophytes and do not occur within the tissues.

The yellow colonies were mostly of one kind, judged by their appearance on agar plates—round, smooth, shining, lemon-yellow with entire margins—and they appeared on the potato agar in from one to two days. This type of colony was chosen for inoculation experiments.

The white colonies of halo-producing organisms from natural infections were all alike on beef-peptone agar, but on potato agar two only of the many isolations gave colonies of a slightly different character, like that designated in this paper as "stock."

On potato agar most of the isolations gave raised, umbonate colonies of a butyrous consistency with thin margins, entire or slightly undulate. This was the usual type of colony isolated. The two varying isolations were from a leaf lesion from Lafayette, Ind., and a glume lesion on Wisconsin No. 14 oats in an experimental plot. (See Pl. 31, C; 32, A.) The colonies were thicker and of an equal thickness out to the margin; the margin was slightly undulate, and the consistency of the colony was like that of boiled starch or gelatin. They gave a more rapid and abundant growth on potato agar than the common type. This second type of colony is the same as an isolation made in 1916 by Mr. Reddy from a halo lesion on oats and kept as a stock culture at Madison, Wis.

The pathogenicity of each of these 28 isolations from natural infections was tested and proved by one or more inoculation experiments. Mr. Reddy's oat stock culture and isolation No. 36 (the common form) from a leaf lesion from Wooster, Ohio, were used as representatives of the two types of white colonies in the inoculation and cultural work and are designated respectively as "stock" and "36."

INOCULATION EXPERIMENTS

1. Inoculation experiments were carried on at Madison, Wis., in experimental plots out of doors and in the greenhouses. The plants in the field were in various stages of development, from half grown to fully headed; and those in the greenhouse were from 4 to 8 inches high. The uninjured plants were sprayed with water suspensions of organisms from agar slants 2 days to 1 week old. The greenhouse plants were then placed in damp chambers for 48 hours. Plants sprayed in the field were covered with water-proofed translucent (glassine) bags for the same length of time. Control plants were sprayed with sterile water and treated in the same manner. Oat plants of Wisconsin No. 1, Wisconsin No. 5, and Wisconsin No. 14 were used for greenhouse inoculations. Wisconsin No. 14 was used more often than the others because it proved to be more susceptible than any other variety. Occasionally halo lesions appeared at the end of the first 48 hours, when the plants were removed from the damp chamber; but usually none appeared until 3 to 4 days after inoculation. On young plants the lesions were often so numerous that centers of infection appeared in rows where the organisms had entered the stomata. The halolike discolorations around these points of sunken tissue were at first only slightly lighter green than the normal tissue but quickly became more marked until about a week after inoculation, when the tissue was a distinct yellowish green

to yellow. Numerous confluent lesions quickly killed the leaf tips and margins, which shriveled, turned brown, and died. Isolated lesions developed in the same way into distinct oval spots of yellow tissue 1 cm. or more in diameter with small dead centers. Infection was always abundant on inoculated oat plants. (See Pl. 27.)

Cultures proved by inoculation experiments to be pathogenic were kept as stock cultures. In this way 21 such cultures were obtained.

2. Since both yellow and white colonies were isolated from leaf sections showing halo lesions, inoculations were made with pure cultures of each and also with mixed cultures of yellow and white colonies for comparison with inoculation work done by Thomas F. Manns (*3, p. 107, Pl. I*). In 25 inoculation experiments pure cultures of the white halo organisms produced abundant and typical infections. In 13 tests, pure cultures of the yellow organisms produced no lesions whatsoever. Twelve sets of inoculations were made with mixed cultures by combining the 2 white halo organisms, No. 36 and stock, with 4 different isolations of yellow organisms. Isolation 39a from a leaf lesion from Urbana, Ill., was the yellow organism most often used. Separate pure cultures of yellow and white organisms were used for control inoculations. The cultures were mixed just before the inoculations were made for the reason that long-continued attempts to grow mixed cultures in broth or on various agars were not successful.[1] In the 12 inoculation tests with the yellow and white mixed cultures typical halo infections were produced, but the lesions were only one-half to three-fourths as abundant as on plants inoculated with pure cultures of the white organisms. The development of lesions from mixed cultures was also somewhat retarded, the infections being evident from one to two days later than those obtained from the pure white cultures. These inoculation experiments showed plainly that the white organism alone is responsible for the production of the halo lesions while the yellow organisms used are neither parasites nor favorable to parasitism.

3. In June, 1918, field inoculations were made on the following 13 Wisconsin varieties: Wisconsin No. 1, 3, 4, 5, 7, 13, 14, 15, 22, 25, 49, 52, and 62. The plants were just beginning to head out, and the experiment was carried on to test the pathogenicity of the white organisms on mature leaves and on panicles, and the effects of possible lesions upon the development of the panicles, spikelets, and kernels. Water suspensions of the halo organisms were sprayed into unopened sheaths upon uninjured bundles of plants, the tops of which were drawn together and tied close so as to be covered with bags, and upon bundles of plants

[1] For two months mixed cultures of white and yellow organisms were grown on potato agar and in +10 beef-peptone broth. Plates poured from these cultures when they were 5 days old showed a few white and many yellow organisms in the broth cultures and about equal numbers of yellow and white on agar. Plates poured from these cultures 7½ weeks later showed no growth of either white or yellow colonies from the agar and showed pure cultures of the yellow organisms from the broth. On the contrary, separate pure cultures of the same organisms held for the same time in these media and under the same conditions gave abundant and characteristic colonies on the plates poured.

injured with a scalpel or drawn between the fingers to rub off the bloom. Bundles of control plants were treated in the same manner and sprayed with sterile water. All inoculated and control plants were covered with glassine bags for 48 hours, as stated above. Characteristic halo lesions appeared on all the varieties inoculated except Wisconsin No. 4. Only uninjured plants of this variety were inoculated. Five other varieties (No. 22, 25, 49, 52, and 62) showed no lesions on uninjured plants, but all varieties showed fairly abundant spotting of leaves and sheaths of plants which had had the bloom removed or had been cut with a scalpel. Some of these leaves were almost entirely yellowed with lesions. On 6 varieties lesions appeared on uninjured plants, but the lesions were not nearly so abundant as on injured leaves and panicles. Wisconsin No. 7 was the only variety in which the panicles were entirely out of the sheaths. In this variety every spikelet of the injured panicles showed halo lesions which stood out as oval yellow spots on the glumes. About half of the spikelets in these panicles were not filled out. Spikelets of untreated panicles of the same variety were also poorly filled out. Under favorable conditions the panicles appear to be just as susceptible to halo-blight as the leaves. Wisconsin No. 14 also showed heavy spotting of injured panicles. Uninjured spikelets of two varieties were halo-spotted when the suspension was sprayed into the unopened sheath.

Though none of the controls showed any halo lesions, both water-sprayed controls and inoculated plants showed considerable sterility, amounting to from one-fifth to one-half of the spikelets in a panicle. Untreated heads of the same varieties and in the same plots showed either no sterility at all or only traces at the base of the panicle. This sterility was particularly abundant when either the water suspension or sterile water was sprayed into unopened sheaths or sheaths just opening at the top. The fact that both controls and inoculated plants showed the same amounts of sterility would indicate that the sterility was not due to the effects of the organism. Excessive moisture around the developing spikelets while these were still inclosed within the sheath offers the most plausible explanation for this sterility. In the same way heavy rains at the time oat fields are heading out probably account for the sterility commonly observed in oat fields. This set of field inoculations has led to the following conclusions:

1. Leaves and panicles of oat plants approaching maturity are susceptible to halo infection under favorable conditions.

2. Infection takes place more readily on injured than on uninjured parts of the plants.

3. Some varieties are more susceptible to infection than others. Greenhouse inoculations on young plants also led to this conclusion.

4. Although both natural and artificial halo infection may occur on heads, these infections are not responsible for the blasting of oat heads. Sterility is due probably to physiological rather than pathological conditions.

CULTURAL CHARACTERS

I.—STOCK HALO ORGANISM

MORPHOLOGY.—The organism is a motile rod with rounded ends (Pl. 34, B, E), sometimes occurring singly or paired, but usually in short to long chains (Pl. 34, C). Organisms grown on beef-peptone agar and potato agar and stained with Ribbert's capsule stain, gentian violet, and carbol fuchsin measure from 1 to 4 μ in length and from 0.4 to 0.8 μ in width, with an average of 0.65 by 2.3 μ. Stained by the Van Ermengen method from 24-hour cultures on beef-peptone agar, the organism shows from one to several polar flagella about the same length as the organism or only a little longer (Pl. 34, E). No spores have been observed, although special staining methods with hot carbol fuchsin and methylene blue were used. Capsules are formed on both potato and beef-peptone agar and were stained with Ribbert's capsule stain (Pl. 34, D). Compact pseudozoogloeae are not formed—that is, there is little or no viscidity. No branched forms have been observed.

NUTRIENT BROTH.—Beef-peptone bouillon (+10) shows light clouding in 24 hours at 25° C. In 5 days there is moderate uniform clouding, and a flocculent white film or pellicle forms on the surface and falls to the bottom of the tube in small white flakes. On further shaking the flakes disappear. In older cultures there may be no pellicle but merely a slight ring around the surface. The clouding is never very heavy, and the thin surface film soon disappears. The medium is gradually changed in color until at the end of 60 days it is a deep amber brown.[1] The odor of decay is distinct with more or less of the penetrating smell of ammonia. The sediment in cultures from recent isolations is loosely flocculent. There was a somewhat viscid swirl in some of the old broths containing sodium chlorid. Rectangular crystals form at the surface.

BROTH PLUS ABSOLUTE ALCOHOL.—To 10 cc. of +15 beef-peptone bouillon absolute alcohol was added to make 4, 5, 6, and 7 per cent. There was heavy clouding in 4 and 5 per cent, moderate clouding in 6 per cent, and slight clouding in two out of three tubes of 7 per cent.

AGAR STROKE.—On +10 beef-peptone agar slants growth in 2 days is moderate, flat, undulate, white, shining, translucent, slightly contoured, butyrous. The medium is slightly browned. There is a slight odor of decay.

On potato-dextrose agar slants the growth in 2 days is abundant, slightly undulate, raised, glistening, smooth, opaque, white, of gelatinous consistency (Pl. 30, B, *b*). The medium is unchanged and there is no odor.

AGAR COLONIES.—(1) On poured plates of +10 [2] beef-peptone agar from +10 broth cultures, colonies appear after 30 hours at 25° C. as tiny translucent dots. When 2 days old the colonies are 1 to 2 mm. in diame-

[1] RIDGWAY, ROBERT. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D.C. 1912.

[2] Fuller's scale.

ter, white, smooth, shining, round, with a denser center. When 11 days old they are more or less irregularly circular, 5 to 10 mm. in diameter in thin-sown plates, and flat with slightly raised margins. Microscopically, with low powers, the internal structure is filamentous, the margin consisting of folded parallel strands or chains. The margin is undulate. Deep colonies are lens-shaped and opaque. The medium may be slightly browned. The markings in Plate 31, A, B, E, F, are characteristic of these colonies under a hand lens, and they do not disappear as the colonies grow older. Very similar markings appear in young colonies of some of the softrot organisms. A half dozen of these softrot organisms tried on oats have not produced any halo lesions.

(2) On plates of potato-dextrose agar the colonies grow more rapidly. They are raised, white, shining, opaque, with only slightly undulate or entire margins, and of the same gelatinous consistency described above (Pl. 31, C; 32, A.)

GELATIN COLONIES.—Growth slow, circular, crateriform, margins entire to undulate with folded strands (Pl. 31, G), liquefaction saucer-shaped, rather slow.

GELATIN STAB.—Growth on +10 peptone gelatin at 22° C. is slow, best at the top, with only a slight filiform growth along line of stab, liquefaction crateriform. At the end of 2 days a small pit is formed at the surface 3 mm. deep. At the end of 6 days liquefaction extends two-thirds of the way across a 20-mm. tube, and the pit is 10 mm. deep. At the end of 18 days liquefaction covers the surface to a depth of 14 mm. At the end of 60 days (at 20°) the tube is only half liquefied.

POTATO CYLINDERS.—At 25° C. there is moderate growth in 24 hours and slight darkening of the medium. At the end of 4 days growth is abundant, flat, smooth, glistening, butyrous to slimy, of a cream color, and the medium is a uniform dark gray color. At the end of 20 days there is a decided odor of decay. There is feeble diastasic action on starch.

SMITH'S POTATO STARCH JELLY (5).—Growth moderate, diastasic action feeble, medium stained a light bluish green. Traces of dextrin.

STARCH AGAR.—To melted tubes of +10 beef-peptone agar sterile potato starch was added and plates poured. Tests with iodin showed no diastasic action.

LITMUS SUGAR AGARS.—One per cent lactose, maltose, dextrose, saccharose, and galactose were used in beef-peptone litmus agar. Change of medium to a bright red showed considerable acid production with dextrose and galactose; with saccharose there was less acid produced; and with lactose and maltose there was no evidence of acid production. Reduction of litmus took place to a slight extent under the streaks with dextrose and maltose.

MILK.—In fresh isolations a soft curd forms in from 5 to 7 days, followed by slow peptonization of the curd, which is completed in from 5

to 6 weeks. In old isolations curd usually is absent. The medium does not become viscid or slimy. The liquid at the top of the tube is sometimes a yellowish green but more often brown. This brown color may be confined to a surface layer a few millimeters deep or may extend throughout the liquid medium. In old tubes the fluid is coffee-colored. It is unlike any color in Ridgway, but is somewhat like his moss brown.

LITMUS MILK.—At room temperature the medium begins to turn slightly blue in 2 days, beginning at the top; and in 5 or 6 days it is frequently stratiform, being deepest blue at the top. Reduction begins at the end of a week, the tubes becoming cream-colored throughout, and clearing at the top or showing reduction only at the bottom of the tube for a depth of 1 cm. or more. There is no curdling, and clearing is complete in 2 weeks. At the end of 2 months the tubes are a deep blue-black and sometimes of a gelatinous consistency. At no time is there any reddening.

METHYLENE BLUE IN MILK.—In fresh isolations reduction begins in 3 days and is completed in 7 days, except for a rim of blue at the top 1 mm. deep. Curdling takes place in 1 week; peptonization begins soon after and is completed in 5 weeks, the clear liquid being yellowish to neuvider green, especially toward the top.

COHN'S SOLUTION.—Growth is very slight, appearing in 24 hours and increasing slightly the second day. In a week clearing begins, and at the end of 3 or 4 weeks there is no clouding and only a little precipitate. Nonfluorescent. No crystals.

USCHINSKY'S SOLUTION.—The medium shows light clouding in 24 hours. In 48 hours a thin flocculent white film has formed over the surface and shakes down in fine particles. In 4 days there is moderate clouding, a slight surface film, and the medium is a pale turtle green. In 2 weeks a heavy white rim has formed around the surface of the liquid. When the cultures are 6 weeks old there is considerable white precipitate—fluid, not viscid—and slides stained in carbol fuchsin show a network of long chains (Pl. 34, C). Fluorescence persists in old cultures.

FERMI'S SOLUTION.—Light clouding occurs in 24 hours. In 4 days there is moderate clouding and a delicate surface film which shakes down in fine flocculent particles. In 4 days as much growth as in Uschinsky. In 2 weeks the clouding is heavy, the medium has a greenish tinge, and there is a heavy white surface pellicle 2 to 3 mm. deep, which shakes down in strings of fine white particles. There is considerable white precipitate—a heavy growth. In 3 weeks the white surface pellicle and the precipitate become cream-colored. No chains are formed. Greening first visible after about 2 weeks. At end of a month surface pellicle and precipitate tan color. Clouding and pellicle twice as abundant as in Uschinsky.

LOEFFLER'S BLOOD SERUM.—Growth moderate, filiform to slightly undulate, flat, glistening, smooth, medium slightly browned beneath the streak. No liquefaction, not even after 2 months.

SOYKA'S RICE MEDIUM.—The growth and medium in all cases except one were cream colored. A culture marked "stock b" turned the medium a buff-pink.

NUTRIENT BROTH PLUS CARBON COMPOUNDS.—To tubes of +10 beef-peptone bouillon 1 per cent asparagin was added and to other tubes 1 per cent asparagin plus 1 per cent dextrose were added. The growth was equally good in both kinds of media. The organisms seem to obtain their carbon as readily from asparagin as from dextrose.

INDOL PRODUCTION.—Feeble or absent in beef-peptone bouillon or 1 per cent peptone water containing 0.5 disodium phosphate and 0.1 magnesium sulphate.

HYDROGEN SULPHID.—Hydrogen sulphid is not produced. Lead acetate paper suspended over broth cultures is not blackened, and the medium is unchanged when streaks are made on lead carbonate agar plates.

AMMONIA PRODUCTION.—Moderate. Made tests with Nessler's reagent.

NITRATE IN NITRATE BROTH.—No gas is produced in fermentation tubes. Nitrates are not reduced. Tests were made at the end of 9 days and at the end of 2 months.

TEMPERATURE RELATIONS.—The maximum temperature for growth, tested on beef broth and on agar and potato, is 31° C. The minimum temperature for growth is below 0°. Tubes surrounded with ice showed clouding. The optimum temperature for growth is 24° to 25°. The thermal death point is between 47° and 48°.

MOISTURE RELATIONS.—The organisms are very readily killed by drying. Smears were made from 5-day-old broth cultures to sterile cover glasses and placed in sterile Petri dishes. Pieces of these cover glasses transferred to sterile bouillon after 3 hours showed growth in all. All were dead at end of 24 hours. In a repetition, transfers after 6 hours gave no growth.

FERMENTATION TESTS: (1) POTATO JUICE.—Undiluted potato juice was expressed after passing the pared tubers through a meat grinder. Moderate clouding in open arm of fermentation tubes. No growth in closed arm and no gas.

(2) MILK.—At the end of a week the milk at the open end had cleared without evident curdling. Two days later the milk in the closed end had curdled. This curd was gradually peptonized, about a third of it remaining at the end of 2 months. The cleared liquid in the open arm was browned—a chestnut to auburn brown at the surface and gradually changing to a lighter shade through the open arm and a third of the way up the closed arm. No gas was formed.

(3) CARBON COMPOUNDS.—Tests were made in the fermentation tubes with 2 per cent solutions of dextrose, saccharose, maltose, lactose, mannit, glycerin, and levulose in 2 per cent water solutions of Difco's and Witte's peptones. *Bacillus coli* Escherich was used as a control and produced gas in the closed arm. The oat organism produced no gas and

did not grow in the closed arms of tubes containing maltose or lactose. In tubes containing saccharose, glycerin, and mannit there was growth at first only in the open arm, with a sharp line of demarcation between open and closed arms. At the end of a week clouding began to appear in the closed arms of tubes containing these three substances. In 3 weeks there was light clouding throughout the closed arms in saccharose, moderate clouding throughout the closed arms in mannit, and in glycerin heavy clouding to within an inch of the top of the closed arm with light clouding on up to the top. In a later test there was again light clouding throughout the closed arms of tubes containing saccharose. In two later tests a moderate clouding appeared in the closed arms of tubes of saccharose and dextrose in from 4 to 7 days. In a later test of mannit and glycerin there was no clouding in the closed arm. Tests for ammonia with Nessler's reagent gave a positive reaction in solutions of maltose, saccharose, mannit, glycerin, and lactose, but only traces of ammonia or negative reactions in cultures containing dextrose. Titrations with phenolphthalein as an indicator show a higher total titrable acidity in the cultures than in the controls in saccharose and dextrose. These solutions were also acid to litmus as compared with controls in two later experiments. The hydrogen-ion concentrations, determined after about 6 weeks by the colorimetric method, were as follows: Control, $P_H = 4.8$; dextrose, $P_H = 4.8$; maltose, $P_H = 7$; saccharose, $P_H = 6.4$; and lactose, $P_H = 4.8$.

The organisms grew best in saccharose, levulose, and dextrose, showing heavy growth in the open arm and slight to moderate growth in the closed arm. This organism is evidently a facultative anaerobe when certain sugars are available.

TOLERATION OF ACIDS.—Transfers were made to tubes of +10 beef-peptone broth containing 0.1 per cent and 0.2 per cent of citric, tartaric, and malic acids. There was good growth in 0.1 per cent of each acid but only slight growth or none at all in 0.2 per cent.

TOLERATION OF SODIUM CHLORID.—Neutral beef-peptone bouillon containing, respectively, 2, 3, 4, 5, 6, and 7 per cent of sodium chlorid was inoculated from potato agar slants. There was slight clouding of 2 per cent after 3 days. None of the stronger solutions clouded, but slides made from a stringy white precipitate and stained with carbol fuchsin showed that long chains of cells had been formed in all strengths of sodium chlorid. A second test was made, using neutral broth with 0.5, 1, 1.5, 2, 3, and 4 per cent solutions of sodium chlorid and inoculating from broth cultures. There was slight clouding in 1 per cent at the end of 2 days, slight clouding in 1.5 per cent at the end of 3 days, moderate clouding in 1.5 per cent at the end of 5 days. At the end of 7 days there was slight clouding in 2 per cent and moderate clouding and a stringy swirl of precipitate in 2 per cent at the end of 19 days. Stained slides of precipitate from 1.5 and 2 per cent solutions showed a network of long chains. In the second test there was no growth in solutions of more than 2 per cent.

OPTIMUM REACTION AND TOLERATION LIMITS.—Beef-peptone bouillon was adjusted to each of the following reactions with sodium hydroxid and hydrochloric acid +20, +15, +10, +5, 0, −5, −6, −13, −16, and −22. These were uniformly inoculated from broth cultures and kept at 24° C. At the end of 24 hours there was light clouding in −5, 0, +5, and +10. Subsequent clouding occurred in −6 and +15. A stringy precipitate formed in −13, and on −15 a thin surface film developed and the medium was slightly darkened. At the end of 48 hours the clouding in +5 was slightly heavier than in +10, and the flocculent surface film slightly heavier. Clearing began in 3 weeks. At that time +10 was browned, +15 slightly deeper brown, and +5 and −5 showed a greenish tinge. The optimum reaction for growth is, therefore, +5 Fuller's scale, although +10 and +15 are also favorable reactions.

In later tests the limits of growth on agar were +27 and −17, and in bouillon +27 and −18, when the agar was reinoculated from an alkaline culture.

VOLATILE ACIDS.—Tests for volatile acids were negative. Cultures were grown in tap water containing 1 per cent Witte's peptone and 1 per cent dextrose. The steam from these cultures gave an alkaline reaction to litmus although the liquid was acid to litmus.

FREEZING.—Six plates were poured in +15 agar from 24-hour +15 broth cultures. This 24-hour culture was exposed for 1 hour in salt and crushed ice and then six more plates were poured. Eighty-seven per cent of the organisms were killed by this treatment.

EFFECT OF SUNLIGHT.—The organism is sensitive to sunlight; 80 per cent were killed by 15 minutes' exposure on ice in thinly sown beef-peptone agar plates.

VITALITY ON CULTURE MEDIA.—Typical colonies of this organism have been obtained from +10 beef-peptone agar slants which have stood for 11 months and from broth cultures 10 months old. These were tested by inoculation on young oat plants and gave abundant and typical halo lesions.

LOSS OF VIRULENCE.—Loss of virulence on culture media has not been observed in cultures carried for more than 3 years.

GROUP NUMBER.[1]—221.2323023.

The name *Bacterium coronafaciens*, n. sp., is suggested for this organism.

TECHNICAL DESCRIPTION

Bacterium coronafaciens, n. sp.

A motile rod with rounded ends and polar flagella; single, in pairs or long chains, average measurement 2.3 by 0.65 μ; no spores, zoogloea, or involution forms; capsules are formed; slightly facultative anaerobic. On nutrient agar colonies are white, round becoming irregularly circular, flat with slightly raised margins, surface smooth or slightly contoured; deep colonies are lens-shaped and opaque. Its proteolytic

[1] SOCIETY OF AMERICAN BACTERIOLOGISTS. DESCRIPTIVE CHART. Indorsed by the society for general use at the annual meeting Dec. 31, 1914. Prepared by the committee on revision of chart identification of bacterial species.

power is moderate; gelatin is liquefied slowly, beginning in 2 days and not complete in 60 days; reduction of litmus occurs in milk, and the casein is digested without curdling; milk curdles in 5 days, and peptonization is completed in 5 weeks. No acid is produced in milk. Oxidations of proteins are incomplete; ammonia is produced; hydrogen sulphid, gas, and indol are not produced. Nitrates are not reduced. There is slight diastasic action on potato cylinders. Good growth in Uschinsky's solution and in Fermi's solution. Growth in Cohn's solution is scanty. Maximum temperature for growth is 31° C., minimum below 0°, optimum 24° to 25°, thermal death point between 47° and 48°. Tolerates sodium hydroxid to −18 Fuller's scale and hydrochloric acid to +27. The optimum reaction for growth is +5 Fuller's scale. Gram-negative, not acid-fast, stains readily and uniformly with gentian violet and methylene blue. Stains more or less irregularly with carbol fuchsin (often polar staining). Sensitive to drying; 87 per cent killed by freezing, 80 per cent killed by sunlight. Vitality on culture media long. Pathogenic on varieties of cultivated oats and to a slight degree on wheat, rye, and barley, producing oval halolike lesions of chlorotic tissue surrounding dead brown centers of infection.

Beef-peptone agar and beef bouillon are favorable media for prolonged growth. Growth on potato agar brings out more distinguishing characteristics.

II.—ISOLATION NO. 36

This isolation was made from a halo lesion on oats obtained from Wooster, Ohio, in June, 1917. It has the same group number as the stock halo organism just described but differs from it in the characters mentioned below. The differences, though not very marked, seem to be fairly constant, while the lesions from which the cultures were isolated and which they produce in inoculation work can not be distinguished. The stock organism seems to be slightly more virulent.

MORPHOLOGY.—The organism occurs singly or in twos but seldom in long chains (Pl. 34, A). Stained by Ribbert's capsule stain it measures from 1.1 to 3 μ in length and from 0.5 to 0.8 μ, in width, not including the capsule, with an average measurement of 0.66 by 2.1 μ.

BEEF AGAR PLATES.—On +10 beef-peptone agar, the surface colonies remain round, and the margin tends to remain entire (Pl. 31, D).

POTATO-DEXTROSE AGAR STROKE.—Two-day-old slants from broth show moderate flatter growth, which is filiform and dull, with more or less wrinkling on the surface. The growth is somewhat translucent and of a butyrous to slightly membranous consistency (Pl. 30, B, *a*).

GELATIN STAB.—Liquefaction is more rapid, being complete in 40 days.

TOLERATION OF SODIUM CHLORID.—Same as stock, but slides from a 2 per cent solution stained with carbol fuchsin show only a few scattered short chains.

LITMUS MILK.—Litmus is not reduced.

METHYLENE BLUE.—Digestion of casein a little slower than with stock.

USCHINSKY'S SOLUTION.—No chains on slide stained with carbol fuchsin.

COHN'S SOLUTION.—Clouding heavier than with stock. Crystals are formed on the sides of the tubes.

STARCH AGAR.—The organism showed a feeble diastasic action on starch.

TEMPERATURE RELATIONS.—Thermal death point is between 47° and 48° C.

Strain 36 usually gives a greenish tinge to bouillon cultures, which in old cultures contrasts strongly with the brown of old "stock" cultures. On ordinary beef-peptone agar the two strains can not be distinguished but on potato-dextrose agar there is considerable difference in amount of growth, and they are noticeably different in consistency. The most important differences perhaps are in size and in nonformation of chains. The rods of No. 36 are shorter and plumper. They seem to be two strains of the same organism.

III.—YELLOW ORGANISM

MORPHOLOGY.—The organism is a motile rod with rounded ends and one to several polar flagella. It occurs singly or in short chains. When grown for 24 hours on beef-peptone agar and stained by the Duckwall modification of Pitfield method, it has an average measurement of 3.5 by 1.4 μ, varying in length from 2.3 to 3.7 μ, and in width from 0.98 to 2.1 μ. No spores have been found.

BEEF-PEPTONE AGAR PLATES.—Colonies appear after 24 hours on +10 beef-peptone agar; in 2 days they measure 2 mm. in diameter and are a translucent light yellow. When a week old, surface colonies are circular, 4 to 5 mm. in diameter, raised, smooth, lemon-yellow, with entire translucent margins. Microscopically the internal structure is finely granular. Deep colonies are lens-shaped and opaque.

BEEF-PEPTONE AGAR STROKE.—Growth in two days is moderate, filiform, flat, glistening, slightly contoured, translucent, light orange-yellow, with a faint odor. Consistency is butyrous, and medium is unchanged (Pl. 30, B, *c*). The organism lives at least three or four months on beef-peptone agar.

POTATO AGAR STROKE.—In two days the growth is abundant, filiform, flat, spreading, glistening, smooth, opaque, light orange-yellow. The medium is unchanged, and the consistency butyrous.

GELATIN STABS.—At 22° C. growth in +10 nutrient peptone gelatin is moderate. The liquefaction at first is saccate along the stab and later stratiform. Liquefaction is completed in 40 days. The surface growth has a pinkish tinge, but the precipitate is yellow.

BEEF-PEPTONE BROTH.—There is moderate clouding in +10 beef-peptone broth in 24 hours at 25° C., very heavy clouding in 48 hours, and a slight flocculent surface growth. In 3 days there is a heavy membranous pellicle which breaks up when shaken and sinks to the bottom of the tube. The precipitate is abundant and finely granular. Clearing begins in about 2 weeks.

TOLERATION OF SODIUM CHLORID.—Tables of neutral beef-peptone bouillon containing respectively 2, 3, 4, 5, 6, and 7 per cent of sodium chlorid were inoculated from potato agar slants. In 24 hours there was

clouding in 2, 3, 4, and 5 per cent solutions. In 3 days there was a very slight clouding in 6 and 7 per cent solutions. A stringy yellow precipitate formed in the 4, 5, 6, and 7 per cent. Slides made from 2 and 3 per cent solutions and stained with carbol fuchsin showed long chains of cells. There were no long chains in the 4, 5, 6, and 7 per cent. In a second test a delicate pink surface film, not previously observed, formed in 0.5, 1, 1.5, 2, 3, and 4 per cent solutions; and a pink stringy precipitate formed in 2 and 3 per cent, becoming a brick red in 4 per cent.

POTATO CYLINDERS.—At 25° C. there was slight growth in 24 hours and a slight graying of the medium. In 4 days there was abundant yellow growth, and the medium had become slightly browned. Growth was filiform, flat, raised, glistening, somewhat contoured, orange-yellow to red on top. There was no odor, and the consistency was butyrous. There was no action on the starch.

MILK.—Milk titrating +18 on Fuller's scale was inoculated from 9-day-old potato agar slants. A slight yellow surface film was formed in 2 days. At the end of 1 week yellow precipitate was evident. Curdling began in 3 weeks. There was a slight separation of curd and whey at the end of 2 months. The solid curd gradually dried down without any evidence of peptonization.

LITMUS MILK.—Complete reduction occurs in 24 hours, leaving the medium cream-colored. Shaking tended to restore the color. After about a week some of the reduced tubes were steamed, whereupon the original lavender color returned. Curdling occurred in 3 weeks. There was no evidence of digestion at the end of 2 months.

METHYLENE BLUE IN MILK.—Reduction takes place in 24 hours. In 3 weeks there is curdling and the blue color begins to return at the tops of the tubes. No petonization.

USCHINSKY'S SOLUTION.—There is moderate clouding in 24 hours at 25° C. and a membranous surface film. At the end of 2 days there is a fairly heavy light yellow surface film. In 4 days there is heavy clouding and a heavy surface film and yellow precipitate. Slides stained with carbol fuchsin show many short chains.

FERMI'S SOLUTION.—There is moderate clouding in 24 hours at 25° C. In 2 days there is a fairly heavy light yellow surface film. In 4 days the clouding is heavy and there is a heavy orange-colored surface film 2 mm. thick. At the end of a week this pellicle is 4 mm. thick. Clearing begins in 2 weeks, and yellow strands extend from the heavy pellicle to the bottom of the tube. At the end of 3 weeks the pellicle is 1 cm. thick. In 4 weeks the medium has a greenish tinge. No chains were observed on slides stained with carbol fuchsin.

COHN'S SOLUTION.—There is a slight clouding at the end of 2 days at 25° C. At the end of 4 days the clouding is still very light, and there is just a trace of surface growth. Rhomboid crystals are formed on the tube above the liquid. Growth is very slight in comparison with that in Fermi's solution.

BLOOD SERUM.—Growth was moderate, filiform, slightly raised, orange-yellow, smooth, shining. In 2 weeks the center of the growth became red, but the author was unable to verify this change in 1919. The medium was unchanged.

LITMUS SUGAR AGARS.—In 24 hours there is a slight reddening of litmus dextrose agar and in 3 days reduction has begun in the lower end of the tubes, the upper two-thirds being rose red. Litmus-lactose and litmus-maltose agar show reduction in the lower ends of the tubes in 3 days. These tubes are red through the center and blue at the top. At the end of a week all agars are colorless at the bottom of the tubes, red in the center, and blue toward the top. Growth is abundant. At the end of 2 weeks the colony begins to turn red.

STARCH AGAR.—There is no diastasic action on starch.

INDOL.—Indol production is feeble.

NITRATE BOUILLON.—No gas is produced in fermentation tubes. Nitrates are not reduced.

AMMONIA.—Ammonia production is moderate.

HYDROGEN SULPHID.—No hydrogen sulphid is produced. Tests were made with lead-acetate paper over broth and with lead-carbonate agar.

OPTIMUM REACTION AND TOLERATION LIMITS.—By the use of sodium hydroxid and hydrochloric acid, using phenolphthalein as indicator, beef-peptone bouillon was adjusted to each of the following reactions: +25, +20, +15, +5, 0, −5, −6, −13, −15, and −22. These were uniformly inoculated from broth cultures and kept at 24° C. In 24 hours there was clouding in all except +20 and +25. At the end of 3 days there was clouding in all except +25. The clouding in +20 was slight. At the end of 1 week there was no growth in +25, light clouding in +20, −15, and −22, and heavy clouding in all the other reactions, with precipitation and surface growth. In 3 weeks there was clearing in −15 and −22, but a viscid yellow precipitate. There was never any growth in +25. The optimum reaction for growth is +5 Fuller's scale.

GAS FORMATION AND AEROBISM.—Tests were made in fermentation tubes in the presence of the following carbon compounds: dextrose, saccharose, lactose, maltose, mannit, and glycerin. A 2 per cent solution of each was made in a 2 per cent water solution of Difco peptone. *Bacillus coli* Escherich was used as a control and produced gas in each solution. No gas was produced by the yellow organism. There was clouding in the open arm of all tubes in 2 days, the heaviest growth being in saccharose and maltose. In 3 days clouding began in the closed arm of tubes containing saccharose and mannit. At the end of a week there was clouding in the closed arm of all tubes—heavy in glycerin and mannit, light in dextrose, and moderate in the others. Tests for ammonia with Nessler's reagent gave a positive reaction in all sugars—slight in glycerin, and moderate in the others. Titrations with phenolphthalein as indicator showed no acid production. The hydrogen-ion concentrations were

determined by the colorimetric method at the end of 6 weeks. The P_H for dextrose was for maltose 5 to 5.2, for saccharose 4.6, for lactrose 7, for glycerin 4.8, and for mannit 6. Controls and *Bacillus coli* Escherich showed a P_H of 4.8 throughout.

TEMPERATURE RELATIONS.—The maximum temperature for growth is above 38° C. The minimum temperature for growth is 3°. The optimum temperature for growth is 24° to 25°. The thermal death point is 48° to 50°. Tests were made by the same methods as those used for the halo organisms.

VITALITY ON CULTURE MEDIA.—The organism lives for 2 months on beef-peptone agar at room temperatures. It is nonpathogenic.

GROUP NUMBER.—The group number is 221.3333533, according to the descriptive chart of the Society of American Bacteriologists.

TECHINAL DESCRIPTION

A motile rod, with rounded ends, one polar flagellum or several, single or occasionally in short chains; average measurement 3.5 by 1.4 μ; no spores, pseudozoogloeæ, or involution forms; facultative anaerobic. On beef-peptone agar the colonies are round, raised, smooth, lemon-yellow with entire translucent margins; deep colonies, lens-shaped and opaque. Liquefaction of gelatin begins in 2 days and is complete in 40 days. There is reduction in litmus milk in 24 hours and delayed curdling without subsequent peptonization; milk is curdled in 3 weeks without subsequent peptonization; ammonia production moderate; indol production feeble; does not produce hydrogen sulphid or other gas; no diastasic action on starch; grows moderately in Uschinsky's solution, and very copiously in Fermi's solution. Growth slight in Cohn's solution. Maximum temperature for growth is above 38° C., minimum 3°, optimum 24 to 25°, thermal death point 48° to 50°. Tolerates sodium hydroxid to below −22 Fuller's scale, and hydrochloric acid to +20 Fuller's scale. The optimum reaction for growth is +5 Fuller's scale. Gram-negative; not acid-fast; stains readily with carbol fuchsin, gentian violet, and methylene blue. Nonpathogenic to oats.

OVERWINTERING AND DISSEMINATION

There is evidence from three sources that the organism causing halo-blight winters over on the seed: (1) the presence of typical halo lesions on the glumes and lemmas of maturing spikelets (Pl. 29); (2) the early appearance of the disease on seedlings grown on soil not previously sown to oats (Pl. 28); and (3) the great difference in amount of blight in oat plots from treated and untreated seed.

(1) NATURAL AND ARTIFICIAL INFECTIONS OF SPIKELETS

In 1918 at the time the oat plants were heading out it became evident from observations of the plot of Wisconsin No. 14 and from artificial inoculation of Wisconsin No. 7 that the spikelets were also susceptible to infection with the halo organism. After the Wisconsin No. 7 plants had headed out a number of uninjured heads were sprayed with a water suspension of the organism. Another bundle of heads, bruised by drawing between the fingers, was similarly sprayed; and both were covered with glassine bags for two days. When the bags were removed infections were already appearing on the bruised spikelets as light green

discolorations on the glumes. A week after inoculation every spikelet of these panicles showed distinct typical halo lesions. Many halo lesions also appeared on the uninjured spikelets. Injured and uninjured controls sprayed with sterile water and treated in a similar way showed no halo lesions.

Early in July natural infections on the spikelets of Wisconsin No. 14 oats were observed. Flag leaves were found which showed either scattered halos or yellow halo tissue the length of the blade and sheath. Where sheaths surrounding the heads were badly haloed, every spikelet in the panicle showed infection. If there is one single lesion on a glume it appears as a typical light green to yellow halo about the point of infection. When the whole glume is infected the tissue becomes yellow and translucent between the veins. Only a few such complete infections of panicles were found. Further observations showed that infections on a small percentage of the spikelets in a panicle were not uncommon even when there were no lesions on the sheaths below. Wind and rain might easily spread the infection directly from lower leaves to panicles. Isolations from the glumes showing these lesions and from the parts inside the glumes gave typical halo organisms. Table I, which records the counts on 42 panicles in one corner of a Wisconsin No. 14 plot, will give some idea of the percentage of blighted spikelets.

TABLE I.—*Number of blighted and blasted spikelets on oats naturally infected with halo-blight*

Panicle No.	Number of spikelets per panicle.	Number of blighted spikelets per panicle.	Number of blasted spikelets per panicle.	Panicle No.	Number of spikelets per panicle.	Number of blighted spikelets per panicle.	Number of blasted spikelets per panicle.
1	58	0	6	24	67	2	17
2	54	0	11	25	33	0	8
3	77	1	5	26	80	0	12
4	66	0	14	27	65	4	14
5	55	0	22	28	45	0	7
6	53	0	12	29	72	0	10
7	55	0	17	30	85	12	1
8	60	0	11	31	46	0	0
9	42	0	14	32	50	25	47
10	64	1	13	33	55	0	8
11	90	2	16	34	45	0	7
12	51	1	9	35	71	29	4
13	61	0	22	36	65	0	14
14	79	36	25	37	109	5	6
15	18	18	23	38	52	0	0
16	87	0	4	39	69	8	8
17	50	0	13	40	66	1	6
18	39	6	21	41	60	3	13
19	54	4	34	42	.....	5	21
20	50	5	35				
21	55	2	14	Total	2,387	165	587
22	62	0	23	Average	59+	4	14+
23	69	0	20	Per cent	.....	6+	24+

In this case 6 per cent of the spikelets are blighted. This accords with the percentage of primary lesions usually observed on seedlings in the field. The sheaths below the panicles numbered 15, 32, and 35 were badly yellowed with halo lesions.

(2) PRIMARY LESIONS ON THE FIRST LEAVES OF SEEDLINGS

These primary lesions have been observed by the writer on more than 30 varieties of oats in Wisconsin in two different years. They may appear as halos on any part of the leaf blade, but they more often occur on the tips or margins of the leaves as shown by Plate 28.

(3) EXPERIMENTS WITH TREATED AND UNTREATED SEED

During the season of 1917 two plots of oats were planted on soil which had not previously been planted to oats. Untreated seed of each of 33 Wisconsin varieties was planted in April, and in May seed of the same 33 varieties was planted after having been soaked for 2½ hours in 1 to 320 formalin (1 pint to 40 gallons). Every one of the 33 varieties from untreated seed showed halo-blight to at least some extent, the amount decreasing as the hot weather came on. Wisconsin No. 14 showed the heaviest blighting, and Wisconsin No. 25 was also heavily spotted. Throughout the season not a single lesion was found on the 33 varieties from treated seed.

In April, 1918, three parallel plots of oats were planted on soil not previously planted to oats. Thirty-three Wisconsin varieties of untreated 1916 seed were planted in the first plot, 44 Wisconsin varieties of untreated 1917 seed were planted in the second plot, and 44 Wisconsin varieties of treated 1917 seed were planted in the third plot. Also treated seed of Wisconsin No. 14 was planted as a fourth plot on the experimental ground where oats were grown in 1917. This seed was treated by soaking for 3 hours previous to planting in 1 to 320 formalin.

Counts of infections appearing in these plots were begun just as the second leaf was coming out. On May 16, 17, and 18 primary lesions were appearing on the first leaves of plots from untreated 1916 and 1917 seed, the number of primary infections varying from less than 1 per cent to 8 per cent in each plot. These primary lesions on the 1916 plot would indicate that the organism may live for two years on the seed. No lesions were found at this time on the plot from 1917 treated seed. Counts were made again in the untreated 1917 plots on May 25, four or five days after heavy driving rains, the normal incubation period for halo lesions. Practically all the first leaves were found to be spotted, and lesions were also appearing on the upper leaves. The condition in the 1916 plot at this time was about the same and continued to parallel that of the 1917 plot. At this same time—9 days after the first appearance of the disease on the untreated plots—scattered halo spots and yellowed leaf tips were beginning to appear on the treated plot, evidently by infection from the

neighboring untreated plots, one of which was only 3 feet away. On May 24 and 25 there were more driving rains, and on the twenty-eighth the effects of these storms were evident. Secondary lesions in the untreated plots were so abundant that no attempt was made to count them. Many of the first leaves were completely yellowed and dead, and lesions on second and third leaves were so numerous that tips, margins, and even whole leaves were becoming yellowed. On varieties where infections were not so abundant the second leaves showed only scattered lesions. On the treated plot the primary lesions were still few, and there was here very striking evidence of the way in which the organism spreads about a center of infection. More or less circular spots of infected plants could be distinguished with the more heavily spotted plants in the center. The amount of infection in this treated plot gradually increased until most of the first and second leaves showed some spotting, but in none of the varieties was there more than half as much blighting as in the untreated plots. In the third treated plot, Wisconsin No. 8 showed only scattered lesions on the lower leaves and none on the upper. In the untreated plot of this variety the lower leaves were practically destroyed and the upper so badly spotted that they showed a yellow-brown color at a distance. There were similar but less marked differences in other varieties. Through June there was very little rain. The amount of blight gradually decreased until at heading time, about the first of July, very few halo lesions could be found, and the upper leaves were practically unspotted.

No halo lesions were observed on the fourth plot from Wisconsin No. 14 treated seed until about the twenty-fifth of the month, when two or three centers of infection began to appear as small yellow spots. These spread rapidly after each rain until one of them stood out as a distinct yellow spot irregularly 5 by 10 feet in diameter. The plants in this spot at heading time were 4 or 5 inches shorter than the more normal plants about them and headed out about a week later. Subsequently scattered lesions occurred on lower leaves throughout the plot and undoubtedly came either from the first infections observed or from the neighboring plots. If these primary infections had been produced by soil organisms they would probably have been much more general. Either sterilization of seed was not complete or else the infection came from the neighboring plots.

An experiment with hot-air treatment of seed gave additional proof that the organism is seed-borne. A plot from Graber oats heated to 100° C. for 30 hours showed no lesions throughout the season. There was not a single spot. The plot from untreated Graber oats showed an abundance of halo lesions through May and June. On every plant there was some spotting and many lower leaves were yellowed and dead.

This early appearance of lesions on seedlings grown on new soil, the appearance of typical halo lesions on the glumes and lemmas of the

developing spikelets from which the halo organism was isolated, and finally, the absence of the disease on plants from sufficiently treated seed all lead to the conclusion that this is a seed-borne disease.

HOSTS OTHER THAN OATS

Field observations and artificial inoculation experiments indicate that the halo-blight organism of oats does not readily infect other hosts. No halo lesions similar to those appearing on oats have been observed in the field on wheat, barley, corn, or timothy. In Jefferson and Dodge Counties, Wis., fields of oats and barley were planted so close together that the plants were intermingled at the margins. In both places the oat plants were heavily spotted with halo lesions, but even where these spotted oat leaves came in contact with the barley leaves not a halo could be found on barley. At Arlington Farm, Va., one halo lesion was found on a rye plant growing among infected oat plants, but no plates were made. The field was half oats and half rye, and although practically all the oat plants were spotted no other lesions could be found on rye.

Six different sets of inoculation experiments were carried on in the greenhouse during the winter of 1917–18 to test the pathogenicity of the halo-blight organism on wheat, rye, barley, and corn. The methods of inoculation were the same as those described above. The organisms used were stock and No. 36. The results are given in Table II.

TABLE II.—*Inoculations on other plants with halo-blight from oats*

Host.	Experiment I, stock.	Experiment II, stock.	Experiment III, stock.	Experiment IV, stock.	Experiment V, stock.	Experiment VI, No. 36.
Wheat	−	−	+	−	−	++
Rye	−	+++	−	−	+	+
Barley	−	+	+	−	−	++
Spelt	−	.....	.....	.....	.....	.....
Corn	−	−	−	−	.....	.....
Oats, Wisconsin 14	+++	+++	+++	+++	+++	+++
Controls	−	−	−	−	−	−

\+ Slight infection. +++ Heavy infection.
++ Moderate infection. − No infection.

Halo lesions were obtained on wheat in two different experiments, in the second of which the halo lesions were not so large but almost as numerous as on oats.

In three out of six experiments halo lesions were produced on rye. In the first, infection was so heavy that there was a general wilting of the leaves. Typical white organisms were isolated from these leaves which on reinoculation produced halo lesions on oats but not on rye.

Halos on barley were obtained in three out of six inoculation experiments. There were eight halos in the first experiment and two in the second. In the third experiment, six leaves had one or more halos.

Reisolation from the first halos gave typical white colonies which on subculture and reinoculation produced halos on barley and oats.

No halos were obtained on corn in four experiments, and no halos were obtained on broom corn in later experiments. Oat plants inoculated at the same ti[redacted] always showed abundant infection. It is evident that the halo-blight organism may attack wheat, rye, and barley to a slight extent; but in Wisconsin, at least, halo lesions in the field rarely, if ever, appear on anything but oats.

VARIETAL SUSCEPTIBILITY

All observed varieties of cultivated oats are attacked by the halo-blight to some extent. Wisconsin No. 14, both in the field and in the greenhouse, is more susceptible than any other variety and shows more lesions in later stages of development, especially on the flag leaf, rachis, and spikelets. Two varieties, Wisconsin No. 13 and Wisconsin No. 15, grown in the fields on either side of Wisconsin No. 14 during 1917, showed considerable resistance. Although leaves of Wisconsin No. 14 were badly spotted, the leaves of Wisconsin No. 13 and 15, which came in contact with them, showed little spotting. In the first plot (from 1916 untreated seed), described above, Wisconsin No. 128 showed only six primary infections, while Wisconsin No. 124 showed 169. In the second plot (from 1917 untreated seed) some varieties showed only slight secondary infections, others moderate, and some heavy infection.

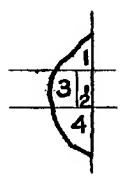
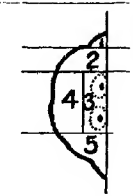
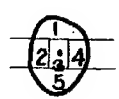
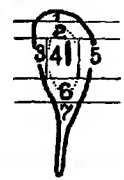
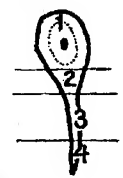
Inoculation experiments in the greenhouse also brought out differences in varietal resistance. Wisconsin No. 1, 5, and 14 were used for several experiments, Wisconsin No. 14 always showing so much heavier infection than either of the other two that Wisconsin No. 1 and 5 were no longer used. Wisconsin No. 1 showed more resistance to infection than Wisconsin No. 5.

While certain varieties are more susceptible than others under ordinary conditions and show fewer primary lesions at the beginning of the season, as above indicated, the differences are not marked in severe blight years as the season advances.

RELATION OF ORGANISM TO HALOED TISSUE

The oval outline of the halo, its rapid spread from the point of infection, and the fact that the haloed tissue remains normal, apparently, except for loss of color, have led to the conclusion that the discoloration is probably due to some diffusible substance produced by the bacteria rather than to their immediate presence. To determine whether or not the bacteria were equally distributed throughout the lesions, isolations were made from pieces of tissue cut from the centers of lesions and from points at varying distances from the center as shown in the following diagrams. Isolations were made after treatment with mercuric chlorid as described above. The distribution of bacteria throughout the halo lesions is shown in Plate 33 and Table III.

TABLE III.—*Results of isolations from sections of halos at different distances from centers of lesions*

	Isolation No. I.				Isolation No. II.					Isolation No. III.					Isolation No. IV.							Isolation No. V.			
																									
Section No.	1	2	3	4	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	6	7	1	2	3	4
Growth in 48 hours	−	+	−	−	−	−	++	−	−	−	−	++	−	−	−	−	−	++	−	−	−	+	−	−	−
Growth in 4 days	−	24	−	−	−	−	++	−	−	−	−	800	−	−	−	1	−	3,600	−	−	−	+	−	−	−

\+ Thin seeding of colonies. ++ Heavy seeding of colonies. − No growth.

The first two lesions used were produced by artificial inoculation. The last three were natural infections from the experimental plots. In all of the five isolations only the plates from the centers of the lesions showed any growth at all, and these plates were heavily seeded with typical white colonies. The only exception is the one colony on a plate from isolation IV, section 2. In isolation I the broth cultures from sections outside the center did not even cloud. In other isolations where broth cultures from sections outside the center clouded, subsequent plates showed that the clouding was sometimes due to the growth of the halo organism and sometimes to contamination.

The bacteria are evidently abundant only in the centers of the lesions, and if any do occur outside in the halo they are very few in number. This indicates that the discoloration of the halo-tissue is due only indirectly to the presence of bacteria, and that some enzym or toxic by-product destroys the chlorophyl. A suggestion of what this by-product might be was obtained from some plates of potato-dextrose agar on which colonies of the blight organism were growing. When colonies of the stock organism were 3 days old distinct halos appeared in the agar about the colonies as illustrated in Plate 32, B. The agar around these colonies was less translucent than that outside the halos and was distinct in outline. These halos in the agar increased in diameter from day to day, showing the concentric circles of growth illustrated in the plate and characteristic of the lesions on oat leaves. Acetic acid dropped on the agar-plate halos cleared them in a minute or two. Drops of ammonium carbonate [$(NH_4)_2CO_3$] and ammonium chlorid [NH_4Cl] on sterile plates of the same potato agar produced in a few minutes halos similar in size and appearance to those produced by the colonies of bacteria. Acetic acid also cleared these halos. Litmus was added to melted potato agar at the rate of one drop of a saturated solution to 10 cc. of the agar, and plates were poured. Streaks of stock were made across the agar as soon as it had hardened. Similar streaks on plain potato agar produced distinct halos about them in two days. In the same time the litmus potato agar had turned a distinct blue for 1 cm. or more on all sides of the growth. It seems probable, therefore, that the ammonia produced by the blight organism is responsible for the destruction of the chlorophyl and for the halolike lesions produced in the oat plants.

Stained sections of haloed leaf tissue also show bacteria only in the center of the lesion. The bacteria at first are intercellular, but later they destroy the cell walls and cause the collapse of the tissue. The collapsed tissue is evident as the dead brown centers of lesions. (See Pl. 35, C.)

COMPARISON WITH OTHER SIMILAR BACTERIAL DISEASES

Seasons of excessive rainfall and of abnormal conditions in the oat fields similar to those of 1918 have been recorded for 1890 and 1907–8. For the earlier record we are indebted to Galloway and Southworth, of the United States Department of Agriculture (*1*), and for the later work to Thomas F. Manns, of the Ohio Experiment Station (*3*).

WORK OF GALLOWAY AND SOUTHWORTH

In 1890 Galloway and Southworth (*1*) published a preliminary note on what they termed "a new and destructive oat disease." This disease appeared in May and June of that season and was so widespread and severe as to threaten to destroy the entire oat crop of the eastern and central States. The signs described were a browning of the tips of the lower leaves, which spread until in a short time all the leaves were dead and brown. Bacteria were found in these lesions. The account of the disease by these authors, however, is too meager to afford any basis for judgment as to whether or not it was the disease here described.

During the seasons of 1906–1909 blade-blight of oats was recorded again over a fairly wide area, and in 1907 it was so severe in some fields as to occasion a loss of from one-half to two-thirds of the crop. In 1908 the blight was threatening at one time but eventually caused little loss. The accounts of the disease from southern Canada and central and eastern States are of the same general kind. They mention a general yellowing of the lower leaves of young plants, the yellow color changing to a brown or red under weather conditions unfavorable to the organism, such as a sudden change from cool, cloudy weather to bright sunshine and higher temperature. The fields are often described as having a rusted appearance because of this reddening of the blades. The trouble was attributed to various causes—to insects, to bacteria, to fungi, and to unfavorable weather conditions.

In 1908 Dr. Erwin F. Smith discovered this disease at Arlington Farm, Va., photographed it, cut sections, and made cultures of the organism on various media, but did not publish upon it nor make any inoculations, although it is quite certain from the type of the disease and the nature of the cultures that he had the same organism here described. This was perhaps its first isolation in pure culture.

No other serious research work was undertaken until Thomas F. Manns carried on his investigations during the seasons of 1908–9 at the Ohio Experiment Station.

WORK OF THOMAS F. MANNS, 1906–1909

Manns (*3*) states that—

> the disease manifests its presence by changes in color varying from a light yellowing, which apparently checks but little the growth of the oats, to a pronounced reddening, which in severe cases kills the blades, leaving only the younger leaves and the central axes alive.

The primary yellowing sooner or later changes to a mottled red or brown. In another place he says:

> The preliminary effects of this disease is a yellowing, beginning either as small, round lesions on the blade, or as long, streak lesions extending throughout the blade or even the whole length of the culm and blade. Occasionally it begins at the tips and works back into the culm; again the upper leaves often break down through a weakened condition of the plant from defoliation below.

When lesions work back from the leaves to the culm a general yellowing and collapse of all the foliage may result. In 1909—

> the disease in the majority of infected leaves began as small yellow spots on different parts of the blades. When these points of infection were numerous, the infected areas quickly became confluent, and the collapsed leaf showed a brownish mottled appearance.

These brief statements are the only references in the bulletin (exclusive of Pl. XIII) to anything at all corresponding to the lesions characteristic of the blight here described, and there is much that is contradictory. His colored figures as well as most of his text indicate an entirely different disease, but his Plate XIII shows that this halo-disease formed at least a part of the phenomenon under consideration. The distinct reddening which he describes and which he illustrates in Plates X and XI was not observed anywhere in Wisconsin even in the worst blight year, 1918. A distinct reddening of oat leaves was observed in our plots but was not due to the halo-blight. Two unsuccessful attempts were made by the writer to isolate bacteria from these reddened leaves. Manns attributes the severity of the outbreak in 1907 to the abnormally low temperatures of April, May, June, and July and to the unusual amount of rainfall during those months and gives convincing climatological data in support of his conclusion. He states that the results of artificial inoculation in the greenhouse also support this theory that cool, humid weather conditions favor the disease.

Through isolation and inoculation experiments Manns came to the conclusion that the blade-blight of oats was due to two species of bacteria living in symbiotic relations within the host tissue (*Pseudomonas avenae* Manns and *Bacillus avenae* Manns). His isolations were made by sterilizing the blades in 2 to 1,000 mercuric chlorid solutions for 1 to 1½ minutes and following this by four washings in sterile water. He states that in practically all isolations from diseased oats these two bacteria were found to be more or less abundant, and when occurring together they could be plainly seen on the agar poured plates in from 2 to 3 days. The yellow organism (*Bacillus avenae* Manns) always appeared first. As a rule, the white organism predominated.

Inoculations were made by Manns in several ways: (1) Directly from crushed leaves; (2) by hypodermic injection, using separate pure cultures of the white and the yellow organism; (3) by hypodermic injection, using the two cultures mixed (*3, Pl. X*); (4) by spraying mixed

cultures on injured and uninjured leaves; (5) by root inoculations without wounds, using mixtures of the two organisms; and (6) by means of grain aphids.

He reports that inoculations in the field and in the greenhouse showed that the yellow organism when used alone produced no lesions and that the white organism when used alone produced only "limited and nontypical lesions," which formed slowly, extended from ½ to 1 inch from the point of infection, and then remained checked. When a mixture of the two organisms was used the lesions appeared in from 10 to 12 days and spread rapidly. From these results he concludes that the disease is a symbiosis, the white organism requiring the presence of the yellow organism to be actively pathogenic.

He also states that the virulence and viability of the white organism on artificial culture media depend greatly upon association with the yellow organism and that the pathogenic action of the white organism was more marked when carried over winter in mixed culture with the yellow organism than when carried over separately. After nine months in pure culture the white organism failed in several instances to grow.

Manns states that endospores occur. These were stained with hot carbol fuchsin from 2-months-old cultures. The figure of these spores in his Plate IX is too indistinct to be of any value in verifying his statement.

His white organism is described as a short motile rod with polar flagella. These are three to five times the length of the rods in his Plate IX, fig. 4, and one to six times those in his text figure No. 1. The rods measure in the majority of cases 0.75 by 1.5 μ. They are rarely in chains of three to four.

The thermal death point is 60° C. The optimum temperature is 20° to 30°. He states that his organism is pathogenic on oats, corn, timothy, barley, wheat, and bluegrass.

The group number for his white organism is given as 111.2223032. Manns' yellow organism is a bacillus with the group number 222.2223532.

Manns suggests the probability of the organism's wintering over in the soil and so being distributed to the leaves by spattering rains. He states that there is no doubt that on seedlings lesions sometimes start on the roots or on that part of the stem in contact with the soil. He does not describe these lesions. The possibility that the disease is seedborne is not mentioned.

Manns' descriptions of individual lesions are so meager and his descriptions of general signs so inclusive as to lead to grave doubt about his having worked with a single bacterial disease. There is no doubt, however, that he sometimes had typical halo-blight lesions, because of his Plate XIII, but with this exception there is no conclusive evidence from either his text or figures that he had this disease under observation; and the

result of his inoculations as indicated on his colored plates is quite contradictory.

The chief differences between the two white organisms *Pseudomonas avenae* Manns and *Bacterium coronafaciens*, n. sp., are summarized below:

PSEUDOMONAS AVENAE MANNS.	BACTERIUM CORONAFACIENS, N. SP.
1. Produces typical blight lesions only when used with *Bacillus avenae* Manns (a yellow organism).	1. Produces typical halo-blight lesions when used in pure culture.
2. Spreads throughout the lesion when used alone.	2. Found only about the point of infection and not throughout the halo.
3. Virulence and viability on artificial media dependent upon association with *Bacillus avenae* Manns.	3. Virulence and viability not dependent on another organism.
4. Viability and virulence greatly reduced by a number of transfers.	4. Viability and virulence not reduced by transfer.
5. Growth feeble on artificial media. (See *3, Pl. VIII, fig. 3.*)	5. Growth abundant on artificial media. (See Pl. 30, A, B, *a*, *b*.)
6. Liquefaction of gelatin stabs begins in 7 to 12 days.	6. Liquefaction begins in 3 days.
7. Pitting of gelatin colonies begins in 7 days.	7. Pitting begins in 3 days.
8. Visible growth in broth in 3 days.	8. Visible growth in 1 day.
9. Manns does not record browning of broth or other media.	9. Broth and other media turned brown.
10. Milk not coagulated in 30 days.	10. Milk usually coagulated in 5 to 7 days.
11. Acid to litmus milk.	11. Alkaline to litmus milk.
12. No reduction of litmus milk recorded.	12. Litmus milk reduced.
13. Strictly aerobic.	13. Facultative anaerobic.
14. No ammonia produced.	14. Ammonia produced.
15. Nitrates reduced.	15. Nitrates not reduced.
16. Limits of growth, -5 to $+15$.	16. Limits of growth, -18 to $+27$.
17. Thermal death point 60° C.	17. Thermal death point 47° to 48° C.
18. Internal structure of agar colonies amorphous.	18. Internal structure of agar colonies not amorphous. (See Pl. 31.)
19. In hanging drop there are few motile organisms.	19. Active motile organisms in hanging drop.
20. Growth viscid on agar.	20. Growth butyrous.
21. Produces clostridium forms in one week on nutrient glucose agar.	21. No clostridium forms observed in any medium.
22. Produces endospores.	22. Does not produce endospores.
23. Does not form long chains.	23. Forms chains and long filaments.
24. Shorter and thicker than *Bacterium coronafaciens*. Average size 0.75 by 1.5 μ.	24. Average size 0.65 by 2.3 μ.
25. Lives over in the soil.	25. Lives over winter on the seed.
26. Pathogenic on oats, corn, timothy, barley, wheat, and bluegrass.	26. Pathogenic on oats, barley, wheat, and rye.
Group number 111.2223032.	Group number 221.2323023

A bacterial disease producing lesions similar to those of the halo-blight of oats has been described from tobacco (*10*). The lesions are similar to

the halos of oats in that they form "circular chlorotic areas" 2 to 3 cm. in diameter with minute brown centers. The oat lesions, however, have no water-soaked borders, and the affected tissues do not fall out as in tobacco wildfire. A white organism has been isolated from these lesions which differs from the halo-blight organism in the points mentioned below:

HALO-BLIGHT ORGANISM.	TOBACCO ORGANISM.
One to several polar flagella.	One polar flagellum.
Single to long chains.	Single to chains of five elements.
2.3 by 0.65 μ.	3.3 by 1.2 μ.
Capsules.	No capsules.
Odor in agar stroke.	No odor in agar stroke.
Casein not precipitated in litmus milk.	Casein precipitated in litmus milk.
Ammonia produced.	Ammonia not produced.
Thermal death point 47° to 48° C.	Thermal death point 65° C.

The halo lesion so characteristic of this oat disease does not occur in the blackchaff disease of wheat (*6–9*) or the bacterial blight of barley (*2*), while the oat disease lacks the translucent water-soaked stripes of these diseases as well as the exudate so abundant in both. R. H. Rosen has recently published a preliminary note on a bacterial disease of foxtail (*4*), which he thinks may be similar to the halo-blight of oats. His description of lesions as dark brown spots or streaks, however, makes it probable that if it is similar to either bacterial disease of oats it would resemble stripe-blight rather than halo-blight. The writer has not observed halo lesions on foxtail and in two sets of field inoculations has obtained no infections on foxtail with the halo organism.

CONTROL MEASURES

The evidence that the halo-blight of oats is seed-borne seems conclusive. However, no practical method of seed treatment has, as yet, been found which will entirely control the disease. Treatment with formalin for smut controls halo-blight to a marked extent but not entirely. In 1917, treated seed of 33 Wisconsin varieties did not show a halo lesion throughout the season, while the same untreated varieties all showed some halo-blight. In 1918, 44 treated varieties of Wisconsin oats developed primary lesions which, however, were later and fewer than on the same untreated varieties. Even when the blight was most severe it was only about half as heavy in the treated plots as in the untreated. The plot from Wisconsin No. 14 treated seed showed very few primary lesions and little secondary spotting except in patches about these primary lesions. This would indicate that soaking for three hours in 1 to 320 formalin kills many but not all of the organisms on the seed. In Jefferson County, Wis., where most of the seed was treated for smut, the blight during the 1918 season was much less abundant than in Dodge County, where seed treatment was not general.

Another method of seed treatment is being developed at Wisconsin which in 1918 entirely controlled halo-blight. The treated seed was heated in a gas oven at 100° C. for 30 hours. The plot from this treated seed did not show a single halo lesion even during the time when other oats were most severely attacked. The plot from untreated seed of the same variety showed primary infections on 10 per cent of the plants and 100 per cent secondary infections on the lower leaves during May and the first two weeks in June. While oats in good condition successfully withstand this treatment of 30 hours at 100° C., a similar treatment for a shorter period would perhaps be just as effective. The commercial application of this treatment has not as yet been worked out.

SUMMARY

A bacterial disease known as halo-blight was unusually severe in its attack on oats throughout Wisconsin during the 1918 season, and reports of a similar disease were received from southern Minnesota, Iowa, northern Illinois, and Indiana. Such epidemics occur under particularly favorable weather conditions, disappearing with the advent of weather conditions more favorable to the development of the host plant.

Typical lesions of halo-blight are characterized by halolike margins of chlorotic tissue about a center of dead tissue.

Isolations from these lesions have constantly given a typical white organism. Yellow organisms also appear from isolations when the surface of the tissue has not been sterilized.

Inoculation experiments have shown conclusively that the white organism alone is responsible for the production of typical lesions. The yellow organism is evidently a surface saprophyte.

Since few if any organisms are found outside the central infection area, the halo is thought to be due to a diffusible substance, probably ammonia.

The organisms live over winter on the seed, producing primary lesions on the first leaves of seedlings. From these lesions the organisms are carried to other leaves by wind and rain.

It seems probable that the percentage of blasting on oat panicles varies with the severity of the halo-blight from season to season. This blasting seems to be due to the same unfavorable weather conditions which favor the development of the bacterial blight rather than to the disease itself.

Halo-blight lesions from natural infections have never been observed on any hosts except oats and rye. Artificial inoculations show that the halo organism may be slightly pathogenic on wheat, rye, and barley.

When the halo-blight is not too severe, different varieties of oats show differences in susceptibility to the disease.

The organism isolated and described by the writer has the group number 221.2323023. No other white organism used by the writer has produced anything similar to the halo lesions. Other white organisms have in fact produced no lesions on oats. Three strains of softrot organisms with internal markings very much like those of oat colonies have been used, and also the white organism, *Bacterium atrofaciens* McC., which produces lesions on wheat. The name of *Bacterium coronafaciens* n. sp. is suggested for this white halo-producing organism.

Treatment with 1 to 320 formalin, as for smut, checks but does not entirely control the disease. A hot-air treatment for 30 hours at 100° C. does control the blight.

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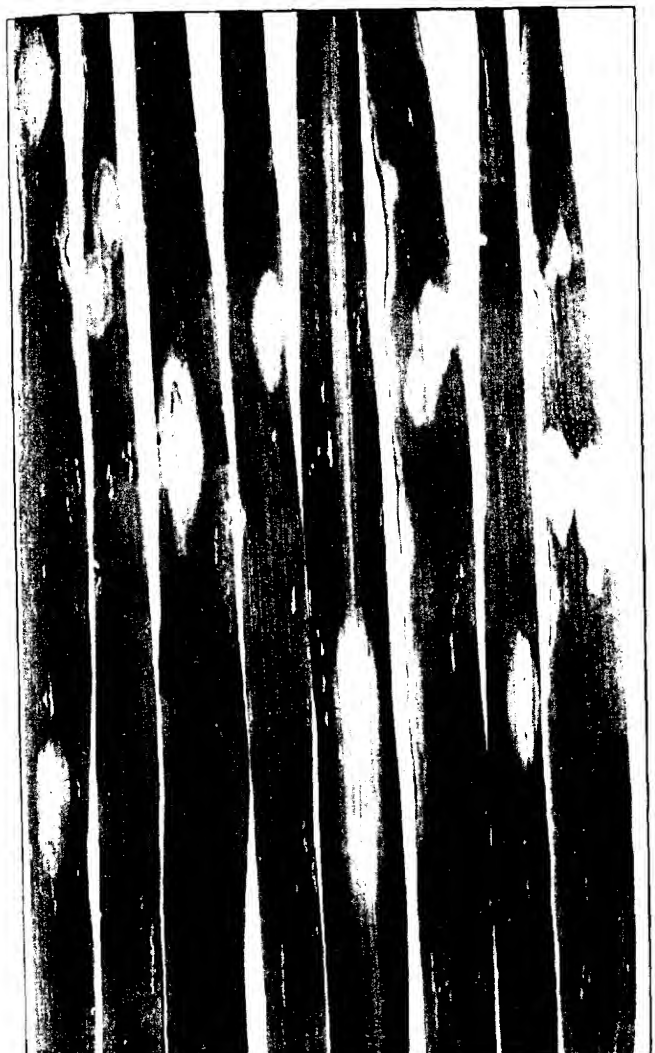
PLATE C

Halo lesions on flag leaves of Wisconsin No. 14 oats. Natural infections from Hill Farm, Madison, Wis. Photographed June, 1917.

PLATE 26

Typical isolated halo lesions. Natural infection on Graber oats. Photographed June 24, 1918. Natural size.



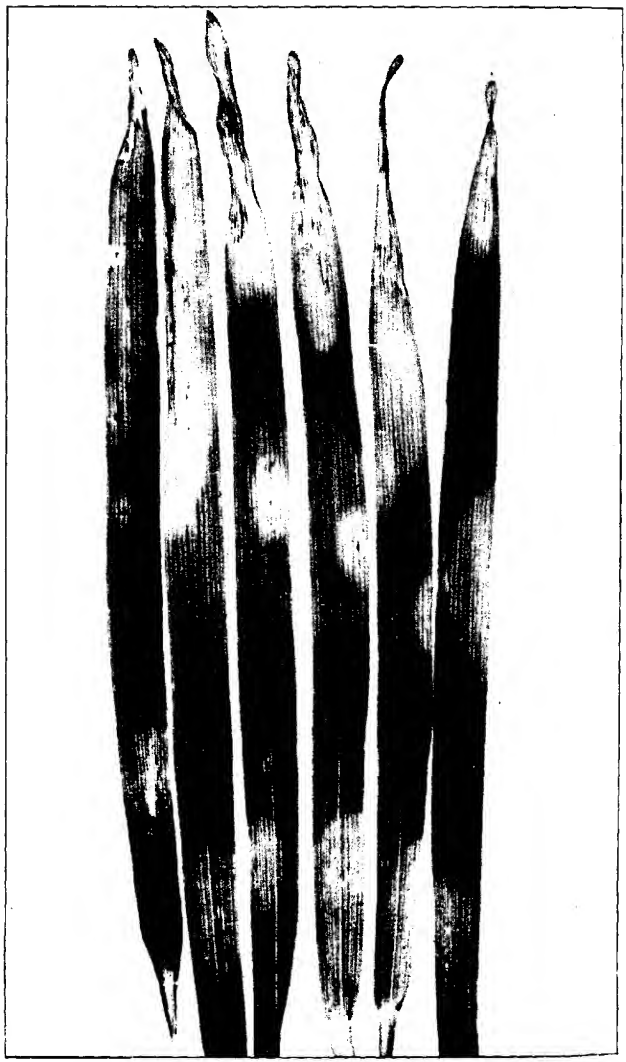
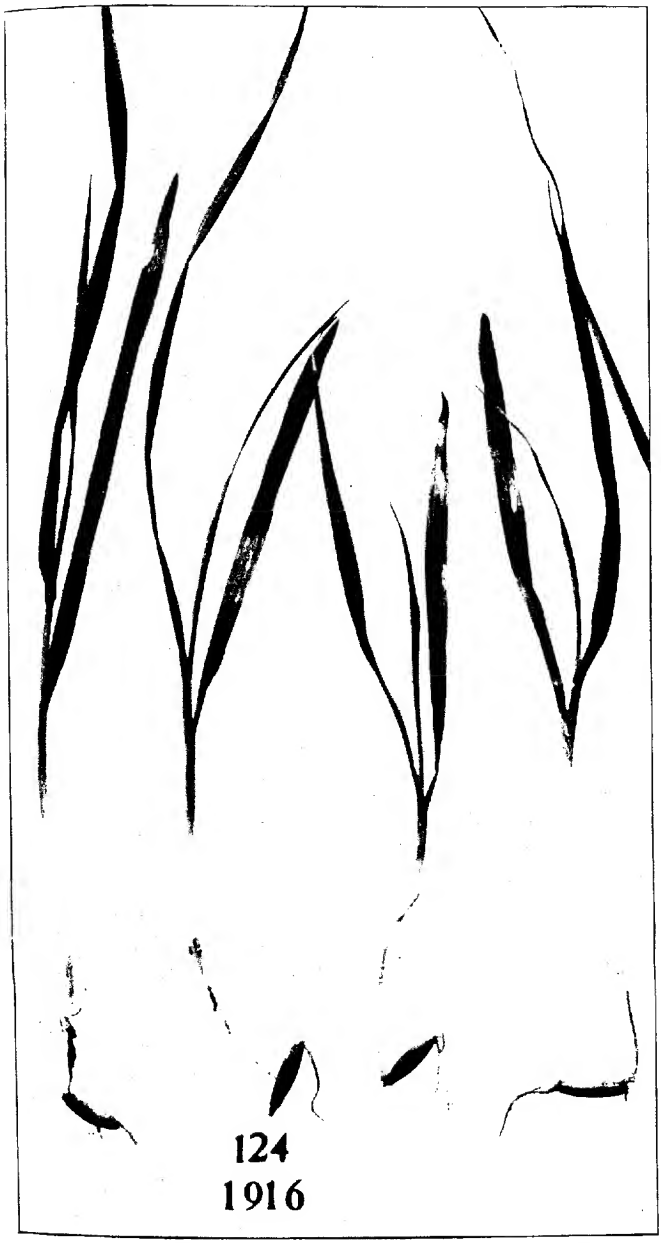


PLATE 27

Halo lesions on Wisconsin No. 14 oats produced by spraying with a water suspension of the stock organism May 26, 1917. Photographed May 31, 1917.

PLATE 28

Infection from untreated 1916 seed of Wisconsin No. 124 oats. Planted April 24, 1918. Photographed May 17, 1918.


124
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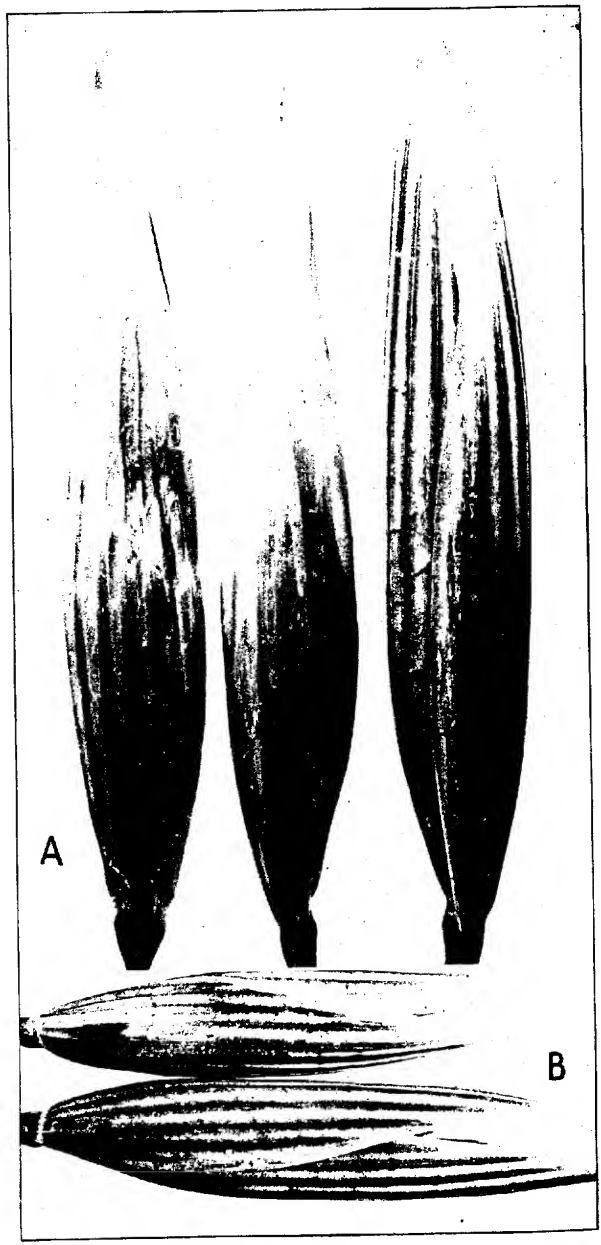

A
B

PLATE 29

Spikelets of Wisconsin No. 14 oats:

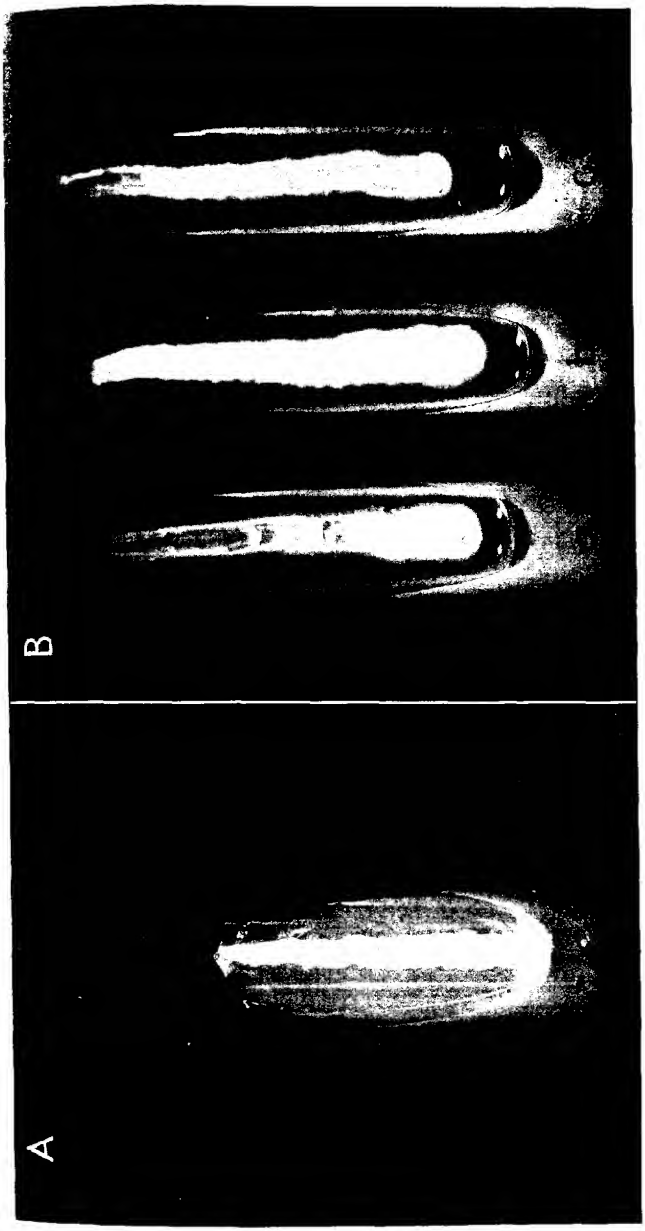
A.—Left and center spikelets show natural infection with halo-blight. Tips yellowed and translucent. Spikelet at right normal, unspotted. Photographed July 17, 1918.

B.—Upper spikelet shows typical isolated halo lesion near base. Lower spikelet normal, unspotted.

PLATE 30

A.—Two per cent +5 glucose Difco peptone beef bouillon agar slant of No. 36. Three-day colony. Photographed August 29, 1919. Natural size.

B.—Two per cent potato-dextrose agar slants. *a*, No. 36, white culture, consistency butyrous; *b*, stock, white culture, consistency of boiled starch; *c*, No. 39a, yellow culture.


B
A

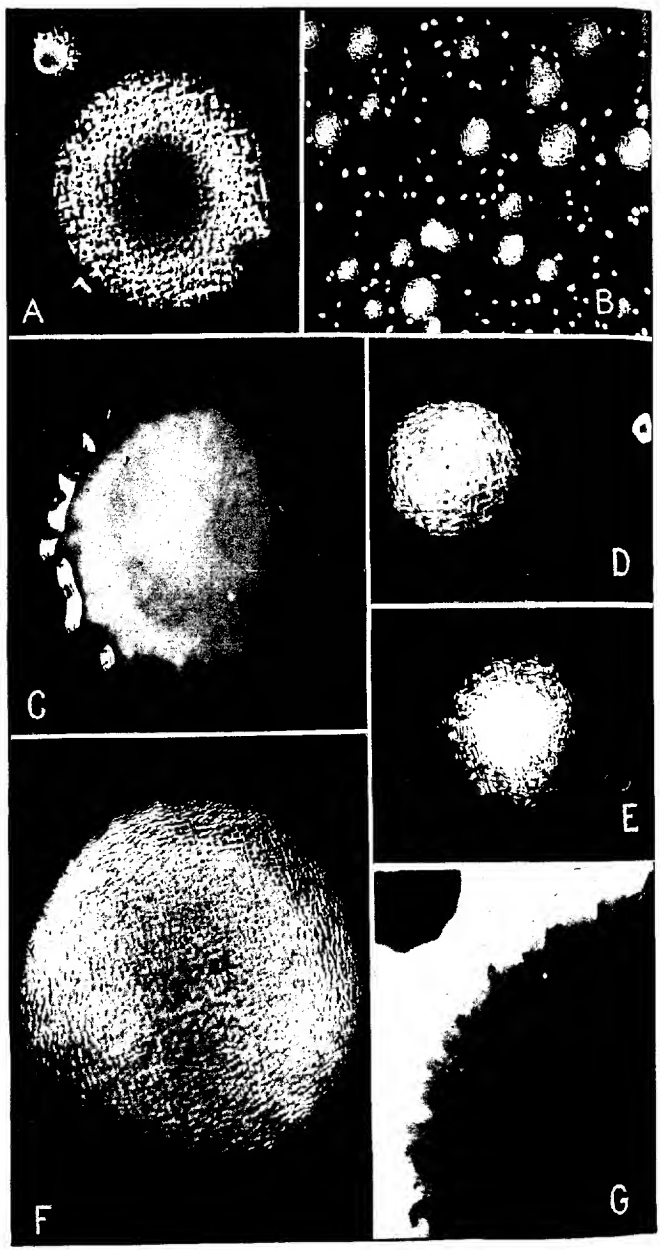

A
B
C
D
E
F
G

PLATE 31

A.—Three-day colony of stock on 2 per cent dextrose-potato agar. Photographed February 17, 1919, by oblique transmitted light. × 10.

B.—Two-day colonies of stock on + 10 beef-peptone agar. Photographed March 26, 1919, by oblique transmitted light. × 10.

C.—Five-day colony of stock on potato-dextrose agar. Colony of boiled starch consistency. Photographed January, 1918, by reflected light. × 7.

D.—Five-day colony of No. 36 on + 10 beef-peptone agar. Photographed October 1, 1918, by oblique transmitted light. × 10.

E.—Three-day colony of stock on 2 per cent glucose Difco peptone beef bouillon agar. Photographed October 7, 1919, by oblique transmitted light. × 10.

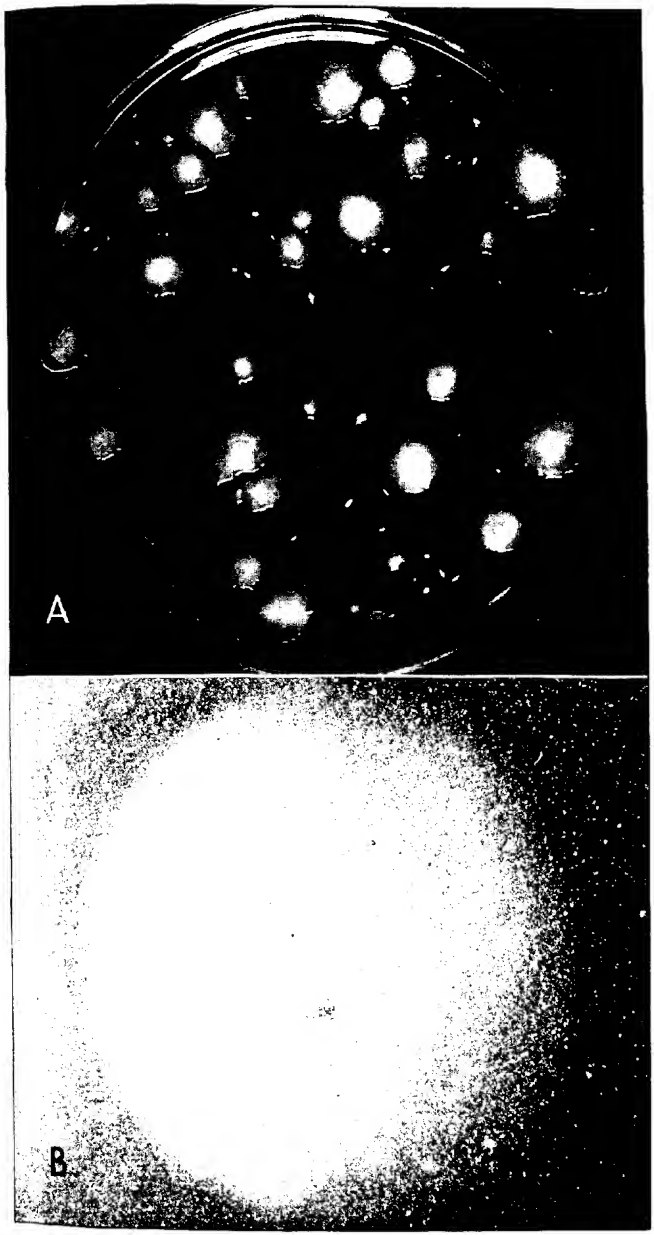
F.—Seven-day colony of stock on + 15 beef-peptone agar. Photographed March 31, 1919, by oblique transmitted light. × 10.

G.—Margin of 3-day colony of stock on + 15 gelatin. Photographed September 30, 1919. × 75.

PLATE 32

A.—Five-day colonies of stock on potato-dextrose agar. Colonies of boiled starch consistency. (For single colony see Pl. 31, C.) Photographed by reflected light. Natural size.

B.—Three-day colony of stock on potato-dextrose agar. Halo about colony. Photographed February 17, 1919, by oblique transmitted light. × 5.


A
B

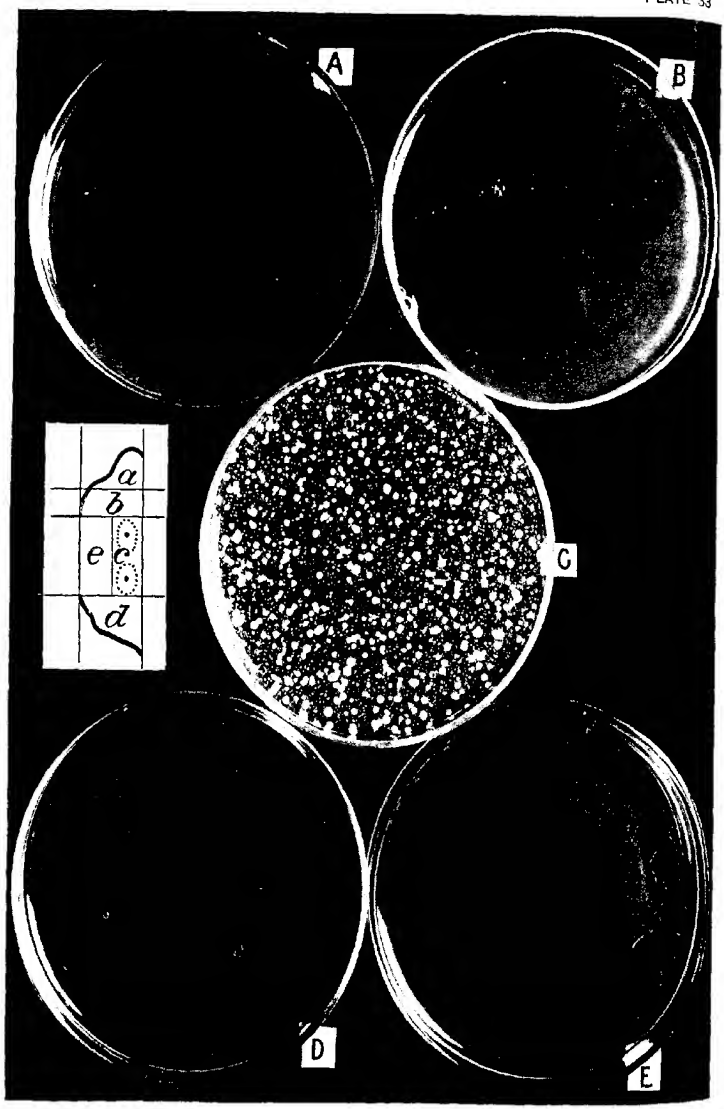

A
B
a
b
e
c
d
C
D
E

PLATE 33

Isolations from sections of halo lesion. The lesion was from an artificial inoculation with oat stock organism made February 25, 1918. Isolations were made March 13, 1918, on potato agar. The sections were dipped in alcohol and then submerged for one minute in 1 to 1,000 mercuric chlorid. The plate from section *c* is the only one showing colonies of bacteria.

A.—Poured plate of isolation from section *a* of lesion.

B.—Poured plate of isolation from section *b* of lesion.

C.—Poured plate of isolation from section *c* of lesion.

D.—Poured plate of isolation from section *d* of lesion.

E.—Poured plate of isolation from section *e* of lesion.

Photographed March 15, 1918.

PLATE 34

A.—No. 36 from 24-hour potato-dextrose agar slant; carbol fuchsin stain. × 620.

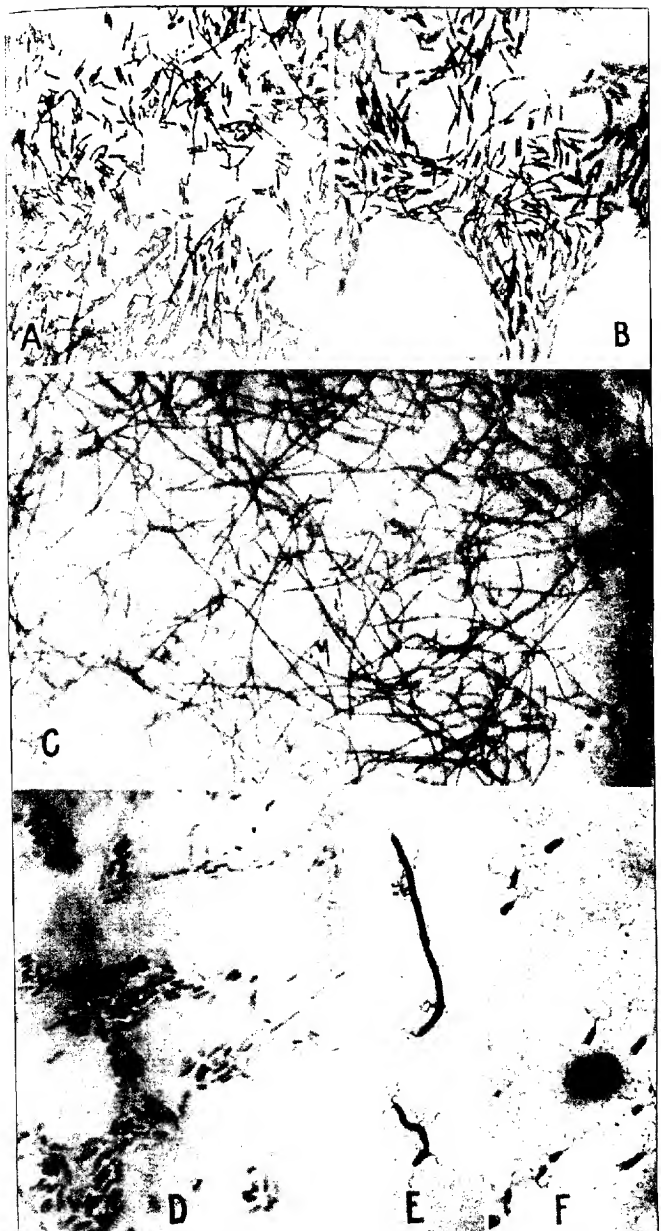
B.—Stock from 24-hour potato-dextrose agar; Ribbert's capsule stain. × 620.

C.—Stock from 4-day potato-dextrose agar; carbol fuchsin stain, showing long chains. × 620.

D.—Stock from 3-day potato-dextrose agar; Ribbert's capsule stain. × 1,550.

E.—Stock from 1-day + 15 beef-peptone agar; Van Ermengem stain. × 1,550.

F.—No. 36 from 1-day + 5 beef-peptone agar; Caesar-Gil stain. × 1,550.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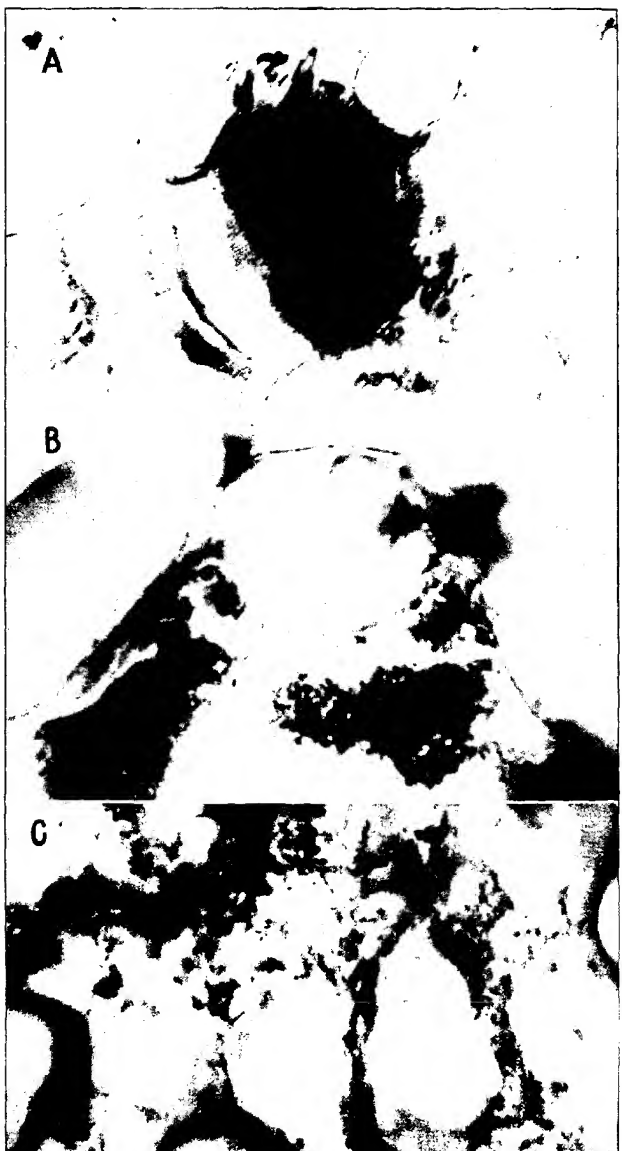
A
B
C

PLATE 35

Sections of oat leaves through halo lesions, showing bacteria in the tissues. Fixed in Gilson's fixative and stained with carbol fuchsin.

A.—Bacteria in substomatal cavity, showing method of entrance of bacteria into the leaf tissue. Cut 15 μ thick. × 700.

B.—Bacteria in substomatal cavity. Cut 15 μ thick. × 1,650.

C.—Section of older lesion, showing bacteria between the cells. In the upper part of this section the tissue is disintegrating at about the point of infection.

Photographed August 26, 1919. × 1,550.

INFLUENCE OF FERMENTATION ON THE STARCH CONTENT OF EXPERIMENTAL SILAGE

By ARTHUR W. DOX, *Chief in Chemistry*, and LESTER YODER, *Assistant Chemist, Iowa Agricultural Experiment Station*

INTRODUCTION

It is not definitely known, though sometimes assumed, that polysaccharoses undergo changes during the formation of silage from green corn. The work reported in this paper was undertaken to determine any changes the starch might undergo together with the nature of these changes and their relation to other important reactions occurring in silage fermentation.

PREVIOUS INVESTIGATIONS

Results of acidity, alcohol, and sugar determinations have been reported by Dox and Neidig (*5*, *6*)[1] and Lamb (*9*) in investigations extending over initial fermentation periods of 30 days and less. Acids and alcohol are rapidly formed at the expense of the sugar, the rate and extent of the changes depending largely upon the nature of the corn.

Babcock and Russell (*2*), Hart and Willaman (*8*), and Esten and Mason (*7*) have made important contributions bearing upon the common changes occurring in silage.

No investigations concerning the starch of corn silage have been reported. Statements are made in an article by E. J. Russell (*13*) of the Rothamsted Station to the effect that bacteria are present which attack the less resistant celluloses and that the disappearance of some less resistant celluloses is a characteristic silage change.

EXPERIMENTAL METHODS

The plan followed by the writers provided for the examination of normal experimental silage at various stages of fermentation. Field corn still green, dented, and about at its glazing stage, cut at about 9 a. m., was taken in bags from the farm silage cutter, brought to the laboratory, and further chopped in the laboratory feed cutter. The chopped silage was then thoroughly mixed, and 2.5 kgm. were packed uniformly into each of 10 wide-mouthed glass jars at 3 p. m. of the same day. The jars were then covered, sealed with paraffin, provided with a valve escape for gases, placed in a large box, and well insulated from exterior temperature influences. On the first day the temperature of the silage rose to 29° C. It remained there for two days, then dropped gradually to room temperature by the seventh day.

[1] Reference is made by number (italic) to "Literature cited," pp. 178-179.

Journal of Agricultural Research,
Washington, D. C.
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Vol. XIX, No. 4
May 15, 1920
Key No. Iowa-7

Immediately after the jars were filled a sample of the original chopped corn was examined. Thereafter a jar of the silage was opened and examined on the second, fourth, sixth, eighth, twelfth, eighteenth, twenty-ninth, forty-fourth, sixty-sixth, and ninetieth days. Determinations were made for moisture, total acidity, alcohol, total sugar, and starch. As a matter of expediency, qualitative tests were made for the transitional products of starch hydrolysis, namely, soluble starch and dextrins.

Although similar data upon total acidity, alcohol, and sugars have been published, this work was repeated because the amount of these products varies so widely in silage made of corn from different sources that correlation with starch changes in this silage would be impracticable. Furthermore, the determinations serve to show that fermentation was normal; and when arranged in series to show changes beyond the first month, they may furnish information not available hitherto.

METHODS OF ANALYSIS

Determinations of constituents soluble in water were made in centrifuged and filtered juice pressed from 2 kgm. of the silage with an hydraulic press.

MOISTURE.—Four hundred gm. of silage were oven-dried at 100° C. to constant weight.

TOTAL ACIDITY.—Twenty-five cc. of silage juice were diluted to volume in a 100-cc. graduated flask with neutral 95 per cent alcohol, mixed and filtered. A 50-cc. aliquot was pipetted into about 300 cc. of neutralized distilled water and titrated with $N/10$ barium hydroxid and phenolphthalein indicator.

ALCOHOL.—The aeration method of Dox and Lamb (4) was used. Twenty-five cc. of silage juice in a 100-cc. cylinder, saturated with ammonium sulphate, were aerated by aspirating air for 24 hours through the alcoholic solution from a dichromate oxidizing solution and through two cylinders, the first containing about 18 cc. and the second about 8 cc. concentrated sulphuric acid. The sulphuric-acid alcohol solution was then transferred to a 500-cc. distilling flask with water free from carbon dioxid, and after the addition of 5 gm. sodium dichromate, it was distilled through a Hopkins trap. The distillate was titrated and the weight of alcohol calculated from its acetic-acid equivalent.

SUGARS.—Determinations were made in preserved samples of the juice. Seventy-five cc. of silage juice were neutralized in a 150-cc. graduated flask with calcium carbonate and made up to volume with absolute alcohol and stored. Of this mixture 100 cc. were diluted to volume in a 250-cc. graduated flask with 95 per cent alcohol. From this point the alcohol extraction method published by Bryan, Given, and Straughn (3) was followed, and sugar was determined by the copper method of Munson and Walker.

STARCH.—After much preliminary work it was found that even by grinding the undried silage in the best grinder available for the purpose a degree of fineness could not be obtained which would give as high results as those secured by drying the silage and then reducing it with a Merker mill till it would pass through a 30-mesh sieve. It was also found that the polarimetric method of Lintner as modified by Porst and Crown (*11*) gave dependable and highly accurate results.

Five gm. of the powdered silage prepared from the residue of the moisture determination were mixed with 20 cc. of water in a mortar and cooled in ice water. To this there were added 40 cc. concentrated hydrochloric acid previously cooled. The mixture was kept at 20° C. for one-half hour. The contents of the mortar were then transferred to a 200-cc. graduated flask with hydrochloric acid of specific gravity 1.125, and 8 cc. of 4 per cent phosphotungstic acid were added. At this point it was found necessary to add charcoal (norite) decolorizer. The mixture in the flask was made up to the mark at 20° with hydrochloric acid of specific gravity 1.125 and kept at 20° for one-half hour. It was then filtered and exactly 15 minutes after filtering (1 hour and 15 minutes after the addition of the 40 cc. concentrated acid) the reading was taken at 20°. From the rotation of 5 gm. pure starch the percentage of starch in the silage was calculated.

Corrections for the zero reading and for optically active substances other than starch were made as follows: A 5-gm. sample was placed in a 200-cc. graduated flask; 100 cc. of 50 per cent alcohol were added; and the whole was boiled for one hour on the steam bath, then cooled and made up to volume with 95 per cent alcohol, mixed and filtered. A 100-cc. portion of the filtrate was evaporated almost to dryness, diluted to about 20 cc. with water, and cooled. The modified Lintner procedure was then followed as outlined above.

QUALITATIVE TESTS.—(1) For soluble starch the test was made by applying the ordinary starch test with iodin to the centrifuged and filtered juice. (2) The dextrin test consisted in adding a sufficient amount of warm saturated solution of barium hydroxid to produce a flocculent precipitate, quickly cooling and filtering, then precipitating the barium in the filtrate with carbon dioxid, refiltering, and adding a slight excess of hydrochloric acid and dilute iodin solution. The presence of dextrins was shown by a red coloration above that of the iodin solution.

EXPERIMENTAL RESULTS

The results were all calculated to the wet basis of the original silage. No correction is made for the specific gravity of the silage juice, since for all practical purposes this error is entirely negligible.

The data for acidity, alcohol, and sugar given in Table I are similar to data obtained by others. A discussion of these is not an object of

this paper except as they relate to starch changes. These results when compared with similar results obtained by previous investigators with silage produced in silos indicated that the silage was normal in every respect.

The silage of each jar examined had a characteristic silage aroma and was free from molds. The fermentation had passed its maximum activity by the eighth day and continued after the first month at a barely appreciable rate.

TABLE I.—*Analysis of experimental silage at different stages of fermentation*

Age of silage.	Moisture.	Total acidity.[a]	Ethyl alcohol.	Total sugar, as invert.	Starch.
Days.	*Per cent.*		*Per cent.*	*Per cent.*	*Per cent.*
0.25	65.56	38.0	0.01	2.94	10.67
2	65.87	126.5	.12	1.84	10.30
4	66.75	211.5	.11	.82	9.93
6	66.00	262.4	.16	.47	10.41
8	66.38	266.6	.10	.55	9.54
12	66.62	279.8	.15	.50	9.62
18	65.63	288.8	.19	.42	10.01
29	66.38	294.7	.26	.32	9.87
44	65.50	291.9	.28	.33	10.77
66	67.12	316.8	.36	.48	9.54
90	66.00	298.3	.39	.48	10.10

[a] Expressed in cubic centimeters of *N*/10 barium hydroxid required to neutralize 100 gm. of silage.

MOISTURE.—Factors usually affecting the moisture content of silage are seepage and excessive respiration accompanied by decomposition of sugar or higher carbohydrates, as studied by Appleman (1) in picked sweetcorn, seepage resulting in a decrease of moisture, and respiration resulting in an increase. Moisture loss by seepage occurs only in silage having an abnormally high water content. The moisture content of the silage in this case was normal and remained constant at about 66 per cent. No excessive decomposition of carbohydrates by respiration is therefore indicated.

TOTAL ACIDITY.—The silage solution, the medium in which fermentation takes place and which is in contact with the silage starch granules, reached a *N*/0.4 concentration by the eighth day and almost *N*/0.5 by the sixty-sixth day. Most of this acidity is due to lactic and acetic acids which are little dissociated and leave after all a small concentration of acid. To bring starch into solution in an acid mixture more or less drastic treatment is necessary; strong acids must be used and their dilute solutions must be heated.

ALCOHOLS.—The formation of alcohol in silage is due to both bacterial growth and enzymic action, their combined effects upon the alcohol production being such that alcohol is not present in uniform quantities

throughout the fermentation period. Appreciable increases in alcohol occurred up to the third month, finally reaching a concentration of 0.39 per cent. That there was no marked maximum production of alcohol at any time was due probably at first to oxidation to acetic acid and later to esterification.

SUGAR.—A maximum loss of sugar from the silage occurred by the sixth day, when the sugar had dropped from 2.94 to 0.47 per cent. After the eighth day the results were quite constant, indicating exhaustion of sugar and the presence of reducing substances which were unfermentable under the conditions existing in this silage. Unless the rate of fermentation equals the rate of formation of sugar no formation of sugar from higher carbohydrates is indicated after the eighth day.

SOLUBLE STARCH AND DEXTRINS.—At no time were positive tests obtained for these products in the silage juice. If they are transitional in the decomposition of starch in the silage, they are so rapidly changed to simpler decomposition products that they are never present in reacting quantities even in green corn. Only in cases of rapid gelatinization of relatively large quantities of starch would tests for these constituents be positive in a medium like that existing in silage. Their absence indicates that the insolubility of the silage starch is the limiting factor in such a series of transitional changes in silage and that no extensive hydrolysis of starch occurred.

Microscopic examination of sections of kernels, leaves, and stems showed no difference in the appearance of the starch granules either with or without stains. No change was discernible in the amount of polarization and in the reaction of the granules with chloral hydrate iodin, enzyms, acids, and alkalies as used by Reichert (*12*) in his chemical differentiation of the starches.

STARCH.—It would have been desirable to include determinations of starch in the undried and fresh silage. Accurate methods for the starch determination, however, require the sample to be in a fine state of division, and such a condition could not be obtained without consequent deterioration of the silage. It was also found that what actually happens when silage is being dried in a drying oven at 100° C. is not gelatinization and hydrolysis with the acids present, as would ordinarily occur in water mixtures of starch at 100°, but rapid desiccation at a temperature below the gelatinization point of corn starch, which is above 65°. The reason for this is apparent from the fact that the evaporating surface is tremendous and the cooling effect due to vaporization is proportional to the amount of water present. When the free water content approaches zero, then the gelatinization and hydrolytic tendencies of starch also approach zero. The partially dried silage gave no positive tests for soluble starch or erythrodextrin, and the sugar content was not greater than that calculated from the determination of sugar in the juice of the fresh silage.

The Lintner method gave almost identical duplicates even when these were run on different days. The variations in the percentages of starch are within 1.23 per cent and are such that no decrease or synthesis of starch is indicated. The lack of consistency in the variations and their correlation with the other fermentation changes gives further evidence that starch is not changed.

SUMMARY

A study of experimental silage at different stages of fermentation which was normal as regards development of aroma and changes in acidity, alcohol, and sugar content leads to the following conclusions:

(1) Changes in total acidity, alcohol, and sugar are entirely independent of the starch content of the ensiled corn and of the silage produced from it.

(2) The first intermediate products resulting from the decomposition of starch are not present in demonstrable quantities.

(3) The starch content remains constant throughout the fermentation process.

(4) The starch granules remain intact, undergoing no physical change that can be detected by microscopic examination.

(5) Since starch constitutes about 10 per cent of the corn plant at the time of ensiling and represents over 400 calories of available energy per kilogram, the fact that no loss occurs during fermentation is an additional argument in favor of silage as an economical feed.

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EFFECT OF PREMATURE FREEZING ON COMPOSITION OF WHEAT

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INTRODUCTION

A consideration of the effects of freezing temperatures upon the chemical composition of the immature wheat kernel is of general interest from a biochemical standpoint and of special interest to those engaged in the study and handling of wheat and its milling products, particularly in the spring-wheat sections. It is of economic importance, especially during the present high prices of wheat and its products, that a large amount of what is popularly called "frosted wheat" is annually classed as fit for nothing better than chicken feed.

This paper presents results of an investigation of the effect of premature freezing on the more important chemical constituents of the wheat kernel, paying special attention to the nitrogen compounds, from which the gluten is formed. Consideration of some of the effects of freezing on the milling and bread-making value of wheat will be taken up in later publications.

Harper (*4*)[2] made some analyses of "rusted and frosted" Minnesota wheats in 1889. He reported that the average protein content for rusted and frosted wheats was more than 2 per cent greater than that for the graded wheats, while the ratio of total nitrogen to albuminoid nitrogen was about the same in the damaged and undamaged wheats. The damaged wheat was higher in ash, fat, and fiber but lower in water and carbohydrates than the sound wheat. Different results of analyses of frozen wheat are reported by Foster and Merrill (*3, p. LXVI*) in connection with some Utah samples. Their figures show the total protein to be about 3 per cent lower in frozen than in sound wheat. The frozen wheat samples contained more fiber, fat, and water than the sound wheat. Shutt (*5, p. 117*), in a report of the analyses of Canadian samples of frozen and sound wheats in 1892, found the frozen samples higher in water, fat, fiber, and ash than the sound samples. The percentages of total nitrogen were very nearly the same in both the sound and frozen wheats. In 1907 Shutt (*6*) determined the albuminoid and nonalbuminoid nitrogen in samples of sound, frosted, and badly frosted wheat

[1] The writer is indebted to Mr. W. F. Day, of the Montana State Grain Laboratory, for milling the wheat samples dealt with in these experiments, and especially to Mr. Edmund Burke, chemist of the Experiment Station, for helpful criticism and advice.

[2] Reference is made by number (italic) to "Literature cited," p. 188.

by the Stutzer method. In badly frosted wheats he found that from 10 to 16 per cent of the nitogen was in the nonalbuminoid form. There was practically no difference in the relative amounts of these constituents in the flours milled from these wheats, a fact which will be discussed later in this paper.

Analyses for the crude food constituents, conducted along the conventional lines, have brought out several facts. Frozen wheat runs higher in fiber, ash, and crude fat than sound wheat, although the differences are not always great. The carbohydrate content of frozen wheat is lower than that of sound wheat. The total nitrogen content may be higher or lower, depending probably on some other factors. The moisture content varies with storage conditions but is undoubtedly greater in frozen wheat at the normal time of cutting than in sound wheat.

EXPERIMENTAL WORK

In order to obtain samples of sound and frozen wheat of the same varieties and grown as nearly as possible under the same conditions, plots were seeded at intervals of a week, starting at the beginning of the normal seeding period and ending about two months later. This insured the likelihood of securing samples frozen at different stages of growth. Marquis wheat was used in the experiments discussed in this paper. A series of plots was seeded in 1917, beginning May 12 and ending June 30. One 1/40-acre plot was seeded each week during the interval, making a total of eight plots. The same procedure was followed in 1918, starting April 29 and ending June 18. Two series of samples were thus obtained. The soil used was a black sandy loam on the grounds of the Montana Agricultural Experiment Station. All plots were irrigated in the middle of July and obviously were not at the same stage of development when irrigated. In all plots the wheat was cut either shortly after maturity or immediately after the first killing frost.

It will be noted that in each series of samples only the last two plots seeded were badly frozen. In the first series the plot seeded last was severely frozen when in the late milk stage, and in the second series the wheat from the corresponding plot was less severely frozen when in the early dough stage. In the two most severely frozen samples a large percentage of the kernels were green, shrunken, and "blistered." These two samples may be considered to represent very extreme cases, and such instances are likely to occur only under the most exceptional climatic conditions. The plots seeded next to the last ones are probably more typical of conditions which are likely to occur in actual farming practice, the one in the 1917 series being more severely frosted than that in 1918. Although it is difficult to measure the exact degree of frosting or freezing in a given sample of wheat between certain limits, these samples present an appearance quite similar to the majority of frosted wheat samples

which habitually come under the observation of the State Grain Laboratory. The kernels were not shrunken, nor was there more than a small percentage of green kernels. The large majority of kernels from the 1917 series, however, had the well-known blistered appearance extending over the entire surface of the kernel, which is usually conclusive evidence that the grain has been badly frozen before reaching maturity. The wheat from the corresponding plot of the 1918 series was much less blistered than that from the 1917 series. All the other samples presented the appearance of mature wheat and had been cut before the first killing frost. The samples just discussed may be readily identified in the tables which follow.

Special attention is invited to the manner in which the grain from each series was handled after cutting, since there is strong evidence from the chemical analyses that the two different methods of handling and storage exerted a very appreciable effect on the biological activities within the kernel, aside from the effects of freezing. The wheat from the 1917 series was brought to the granary shortly after cutting and thrashed when dry enough to permit. Samples for subsequent analyses were then stored in a room near the college heating plant where the temperature was abnormally high and where the grain soon became drier than grain stored under normal conditions. It remained there for more than a year before being analyzed. The grain from the 1918 series, however, was allowed to remain in the field, after it was cut and shocked, until late in the following January, when it was taken to the granary and later thrashed. This grain was therefore subjected to several months of severe weathering in the field, and after being thrashed a considerable portion of it presented the bleached appearance which is characteristic of grain which has stood in the shock and undergone weathering. In the discussion of the analytical results which follow, attention will be called to chemical differences which have apparently been caused by the different methods of handling the grain from the two series of experimental plots.

EFFECT OF FREEZING ON NITROGEN COMPOUNDS

In studying the chemical composition of the wheat frozen at different stages of growth, particular attention was directed to the effect of freezing on the nitrogen compounds, since it is the gluten-forming proteins of wheat that give flour its bread-making power. The influence of the other constituents of normal wheat flour on its baking strength are for the most part considered to be indirect and are of importance only in so far as they affect the gluten. In order to estimate the extent to which premature freezing arrests the building up of the proteins from the less complex nitrogen compounds, determinations of the amounts of total nitrogen, nonprotein nitrogen, a-amino nitrogen, amid nitrogen, and

ammonia nitrogen were made on the respective samples of sound and frozen wheat, as well as on straight flours milled from the wheats. The extraction of the nonprotein nitrogen and its quantitive separation from the proteins, as well as its concentration to enable the estimation of the various forms in which it exists, was satisfactorily carried out by methods previously published by the writer (*2*).

Table I shows the distribution of the various forms of nitrogen in the two series of wheat samples described in preceding paragraphs.

In a previous paper (*2*) it has been shown that while the proteins themselves are completely removed in the method for determining nonprotein nitrogen there still remain some peptids in the solution. Therefore the figures for a-amino nitrogen reported in the tables include the "exposed" a-amino nitrogen of these peptids as well as the a-amino nitrogen of the amino acids. By far the greater part, however, is from the amino acids rather than from the peptids.

It will be noted that the most severely frozen wheat contains two to three times as much total nonprotein nitrogen as the sound wheat. The increase in ammonia and amid nitrogen is proportional to the increase in nonprotein nitrogen, the percentage of these two constituents in terms of the total nonprotein nitrogen remaining practically constant in each series. In the samples of frozen wheat a much larger percentage of the nonprotein nitrogen is in the a-amino form than in the matured samples. In the most severely frozen sample of the 1917 series nine times as much of the total nitrogen of the wheat is in the a-amino form as in the sample which matured earliest.

It is to be noted that the nitrogen in the a-amino and amid forms, as well as total nonprotein nitrogen, runs higher in the mature samples of the 1918 series than in corresponding samples of the 1917 series, while the a-amino nitrogen runs lower in the frozen samples of the 1918 series than in the corresponding samples of the 1917 series. There is much less difference, however, in the figures for total nonprotein nitrogen in the two series, there being nearly the same percentage in the most severely frozen samples of both series. It is strongly suspected that these differences are due to chemical changes caused by allowing the wheat from the 1918 series to stand in the field several months after cutting. Such treatment frequently occurs to Montana wheat in actual farming practice, and its effect on the composition of the kernel will be more thoroughly investigated in the near future, as well as its influence on the baking quality of the flour.

TABLE I.— *Effect of freezing on nitrogen compounds of immature Marquis wheat*

	1917 series, sample No.—				1918 series, sample No.— [a]				
	1.	6.	7.	8.	1300.	1304.	1305.	1306.	1307.
Date seeded	May 12	June 16	June 23	June 30	Apr. 29	May 29	June 3	June 10	June 18.
Date of first killing frost	Oct. 17	Oct. 17	Oct. 17	Oct. 17	Oct. 8	Oct. 8	Oct. 8	Oct. 8	Oct. 8.
Stage of development	Mature	Mature	Immature.	Late milk stage.	Matured but bleached from lying in field after cutting.	Same as 1300.	Same as 1300.	Slightly immature.	Early dough stage.
Percentage of total nitrogen in wheat	2.59	2.67	2.61	2.44	2.59	2.38	2.39	2.17	2.21
Percentage of nonprotein nitrogen in total nitrogen	4.17	4.34	7.05	13.98	7.72	5.88	6.09	10.70	13.20
Percentage of a-amino nitrogen in total nitrogen	.55	.65	1.84	5.05	1.23	1.00	1.07	1.84	3.25
Percentage of a-amino nitrogen in nonprotein nitrogen	13.52	15.18	26.08	36.66	16.00	17.14	17.58	17.24	24.65
Percentage of ammonia nitrogen in nonprotein nitrogen	.....	3.45	3.04	3.28	4.20	2.00	2.74	3.62	4.32
Percentage of amid nitrogen in total nitrogen	.54	.52	.88	1.51	1.10	.80	.90	1.81	1.92
Percentage of amid nitrogen in nonprotein nitrogen	12.96	12.07	12.48	10.82	14.28	14.00	14.96	16.90	14.55

[a] The wheat from the plots harvested in the fall of 1918 was left in the field until late January, 1919, when it was thrashed and stored.

Table II shows analyses of straight flours milled from the more important samples referred to in Table I. The samples in Table II are designated by the same numbers as those in Table I, each number followed by the letter "F."

TABLE II.—*Effect of freezing on nitrogen compounds of straight flour from Marquis wheat*

	1917 series, sample No.—			1918 series, sample No.—		
	1 F.	7 F.	8 F.	1300 F.	1306 F.	1307 F.
Percentage of total nitrogen in flour	2.39	2.46	2.31	2.37	2.11	2.11
Percentage of nonprotein nitrogen in total nitrogen	1.84	4.40	10.56	3.05	3.60	5.12
Percentage of a-amino nitrogen in total nitrogen	.27	1.29	4.85	.57	.53	1.20
Percentage of a-amino nitrogen in nonprotein nitrogen	14.54	29.44	45.90	19.90	14.74	23.33
Percentage of ammonia nitrogen in nonprotein nitrogen	3.20	4.54	2.87	3.40	2.30	2.66
Percentage of amid nitrogen in nonprotein nitrogen	12.73	12.31	10.33	12.64	12.90	10.69
Percentage of amid nitrogen in total nitrogen	.23	.54	1.09	.38	.46	.55

Table II shows that the percentage of total nonprotein nitrogen is in all cases considerably less in the flour than in the whole wheat, although it is much greater in the frozen sample than in the matured ones, especially in the 1917 series. This is not entirely in agreement with the findings of Shutt (6), who used Stutzer's method and reported that flour milled from frosted wheat contained as high a percentage of its total nitrogen in the albuminoid form as flour from sound wheat, although the frozen whole wheat contained a larger percentage in the nonalbuminoid form than did the sound wheat. His conclusion is that the nonalbuminoid nitrogen compounds are practically all removed by the milling process and may therefore be considered to be located in the bran and germ.

The findings of Shutt agree much more closely with the 1918 series than with the 1917 series. Inspection of the figures for total nonprotein nitrogen in Table II shows that a much greater proportion of the nonprotein nitrogen compounds was removed by milling in the 1918 series than in the 1917 series. This indicates that either the freezing was of such a nature that in one season it affected chiefly the nitrogen compounds in the bran and germ, while in the other it affected the whole kernel, or the difference has been caused by the different methods by which the crops from the two series were handled after cutting, as has previously been discussed in this paper.

EFFECT OF FREEZING ON THE CARBOHYDRATES

A brief study of the effects of premature freezing on the carbohydrates of the wheat kernel was made. To this end wheat samples from both series were analyzed by the methods of Stone (7). The results of these analyses are presented in Table III.

TABLE III.—*Some effects of freezing on carbohydrates of Marquis wheat*

	1917 series, sample No. —				1918 series, sample No.—			
	1.	6.	7.	9.	1300.	1305.	1306.	1307.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Reducing sugars	0.02	0.09	0.30	1.86	0.09	0.11	0.16	0.40
Sucrose	1.63	1.35	1.72	1.64	1.65	1.53	1.08	1.27
Dextrin and soluble starch	1.66	1.87	2.10	2.34	1.87	1.75	2.08	2.17

The data in Table III show that the percentage of reducing sugars increases with the severity of the freezing, as would be anticipated. The figures for reducing sugars in the most severely frozen samples of both series offer further evidence that either the freezing in the 1917 series was far more severe than in the 1918 series or the different methods of handling the two series after cutting resulted in different biochemical activities within the kernel. Also, as would be expected, there is more soluble starch and dextrin in the frozen samples than in the matured ones. There seems to be no apparent relationship between the sucrose content and the severity of freezing.

EFFECT OF FREEZING ON ACIDITY

The general effect of freezing on the acidity of the samples of wheat and flour used in these experiments was briefly studied by titrating water extracts with *N*/0.05 alkali, using phenolphthalein, although electrometric titrations with the hydrogen electrode might be preferable. With reference to acidimetric titrations of cereal extracts, Birckner (*1*) has recently shown that the addition of alcohol to water extracts containing amino compounds increases the acidity of the extracts in proportion to the amount of amino compounds present. Water extracts of the wheat and flour samples in question were therefore titrated with and without alcohol. According to Birckner the difference between the two titrations should be an index to the amino compounds present, and a comparison of these differences with results obtained by the use of Van Slyke's microapparatus (see Tables I and II) should be of interest. In the alcoholic titrations the water extracts were diluted with equal volumes of neutral alcohol. The results are set forth in Table IV.

In examining the values expressed by the differences between the titrations with and without alcohol for the respective samples, it may readily be seen that not only do these values increase as the severity of freezing increases but the extent of the increase in almost all instances keeps pace with the figures for nonprotein and a-amino nitrogen as actually determined and shown in Tables I and II. This is in close agreement with the findings of Birckner (*1*).

TABLE IV.—*Acidimetric titrations of wheat and flour extracts with and without alcoho*

[50-cc. portions of water extract used, representing 4 gm. of sample]

	1917 series, sample No.—						1918 series, sample No.—					
	1.	7.	8.	1F.	7F.	8F.	1300.	1306.	1307.	1300 F.	1306 F.	1307 F.
Cubic centimeters of *N*/0.05 sodium hydroxid neutralized without alcohol	3.5	4.6	6.8	1.4	2.0	4.1	3.1	3.8	4.0	1.5	1.1	1.7
Cubic centimeters of *N*/0.05 sodium hydroxid neutralized with alcohol	6.0	8.4	14.4	2.2	4.4	10.0	6.1	7.0	8.8	3.0	2.5	4.0
Difference due to amino compounds	2.5	3.8	7.6	0.8	2.4	5.9	3.0	3.2	4.8	1.5	1.4	2.3
Percentage of nonprotein nitrogen in total nitrogen[a]	4.17	7.05	13.98	1.84	4.40	10.56	7.72	10.70	13.20	3.05	3.60	5.12
Percentage of a-amino nitrogen in nonprotein nitrogen[a]	13.52	26.08	36.06	14.54	29.44	45.90	16.00	17.24	24.65	19.90	14.74	23.33

[a] Figures taken from Tables I and II.

SUMMARY

(1) Premature freezing affects the chemical composition of wheat and the flour milled therefrom.

(2) Frozen wheat contains larger amounts of nonprotein nitrogen, reducing sugars, and acid-reacting constituents than does sound wheat.

(3) The nonprotein nitrogen of frozen wheat carries a considerably higher percentage of a-amino nitrogen than that of sound wheat.

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